

## Effect of iron on the proteolytic activity of live *Uronema marinum* (Ciliata: Scuticociliatida) measured by fluorescence polarization

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Effect of iron on the extracellular proteolytic activity of live *Uronema marinum* was determined by fluorescence polarization (FP) method. Supplementation of 0.5 and 5.0  $\mu\text{M}$  iron significantly increased caseinolytic activity of live *U. marinum*. In contrast, supplementation of 50  $\mu\text{M}$  iron showed no significant differences in FP values compared to the control. The present result suggests that iron in cultured water or skin tissue of olive flounder may influence on the penetration and establishment of *U. marinum*, correlating with modulation of extracellular protease activity of the ciliates.

*Key Words:* *Uronema marinum*, Protease, Iron, Fluorescence polarization

Several scuticociliate species previously regarded as being environmental scavengers, are being recognized as important opportunistic pathogens in marine fish world-widely (Thompson and Moewus, 1964; Cheung *et al.*, 1980; Yoshinaga and Nakazoe, 1993; Dyková and Figueras, 1994; Dragesco *et al.*, 1995; Gill and Callinan, 1997; Munday *et al.*, 1997; Sterud *et al.*, 2000; Iglesias *et al.*, 2001, 2003). In Korea, scuticociliatosis has been one of the major culprits of severe losses of cultured olive flounder, *Paralichthys olivaceus* (Jee *et al.* 2001).

In the pathogenesis of parasitic diseases, proteases have been shown to play important roles in host tissue invasion, digestion of host proteins, and protection against immunological attacks by the host (McKerrow, 1989). As scuticociliates have a high potential for invading systemically and destroying fish tissues (Cheung *et al.*, 1980; Sterud *et al.*, 2000; Jee *et al.*, 2001), proteases excreted by the parasite may play a major role in invasion of host tissues.

Although the infection route of scuticociliates is

not clear, epithelium of gill and/or skin is supposed as a route of infection (Sterud *et al.*, 2000), and iron participates in skin tissue damage through diverse pathological processes (Yeoh-Ellerton and Stacey, 2003). In the present study, we investigated the effect of iron on the proteolytic activity of live *Uronema marinum*, which was identified according to Kim *et al.* (2004), using fluorescence polarization (FP) assay.

The proteolytic activity of the live *U. marinum* was detected by incubating  $5 \times 10^4$  cells of the parasite in 10  $\mu\text{l}$  PBS with 10  $\mu\text{l}$  of 10  $\mu\text{g/ml}$  of fluorescein isothiocyanate (FITC)-casein (Sigma) and 180  $\mu\text{l}$  of PBS (pH 7.0) in a black 96-well plate (Greiner Bio-One Inc., USA) at 25°C.  $\text{FeSO}_4$  was diluted with PBS (pH 7.0) and the final concentrations in 200  $\mu\text{l}$  of a total reaction volume were 0.5, 5.0 and 50  $\mu\text{M}$ . Wells not supplemented with iron were used as controls. In addition, wells with FITC-casein and each concentration of iron without the parasite were used to confirm whether iron influ-

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ence on the fluorescence polarization (FP) value, and wells containing only 200  $\mu\text{l}$  of PBS were designated as blanks.

The FP was measured in a Polarion instrument (TECAN Austria GmbH, Austria). The excitation wavelength was 485 nm and the emission wavelength was 535 nm. All assays were done in triplicate. Readings were automatically recorded at 0, 15, 30, 45, 60, 75 and 90 min in millipolarization units (mP).

The polarization is defined in Eq. (1) where  $I_{vv}$  is the fluorescence intensity when both the excitation and emission polarizers are parallel and  $I_{vh}$  is the fluorescence intensity when the excitation and emission polarizers are perpendicular.

$$P = (I_{vv} - G \cdot I_{vh}) / (I_{vv} + G \cdot I_{vh}) \quad (1)$$

The instrument factor,  $G$ , was set such that a 1 nM solution of FITC yielded a polarization value of 20 mP.

The Student's  $t$ -test was used to determine the statistical difference between each concentration of iron and each relative control. Results were considered significant if  $P < 0.05$ .

The effect of iron on the proteolytic activity of live *U. marinum* is shown in Fig. 1. Supplementa-

tion of 0.5 and 5.0  $\mu\text{M}$  iron significantly increased caseinolytic activity of live *U. marinum*. In contrast, supplementation of 50  $\mu\text{M}$  iron showed no significant differences in FP values compared to the control. Wells containing only FITC-casein and FITC-casein plus iron showed constant FP values during assays.

The gradual drop in fluorescence polarization (FP) values with increase of incubation time indicates that the FITC-casein was cleaved by increasing excreted protease concentrations from live *Uronema marinum*. Fluorescence polarization (FP) assay has the advantage of providing measurements of substrate hydrolysis by live parasites in real-time, which reflect actual conditions more precisely than other protease activity assays including radioactive counting, spectrophotometric or spectrofluorometric analysis and gel electrophoresis. Because measurements were taken in real time, the progress of the reaction was followed both kinetically and at a single point. Additionally, FP assay is homogeneous and, as such, do not require separations, precipitations, or transfers of reaction mixture (Bolger and Checovich, 1994).

Caseinolytic activity of *U. marinum*, in the present results, was significantly increased by iron supplementation. This result suggests that iron participates in the induction of metalloprotease expression. Iron is an essential nutrient for fish as well as microorganisms and the ability of pathogens to infect a host depends on its availability. Maintaining low concentrations of free iron in mucus membranes and in other tissues is thought to be one of the primary nonspecific host defenses against pathogens (Lall, 2000). In humans, iron level increased by skin lesions or ulcers (Yeoh-Ellerton and Stacey, 2003). Recently, Iglesias *et al.* (2003) reported that experimental infection of turbot by *Philasterides dicentrarchi*, a scuticociliate

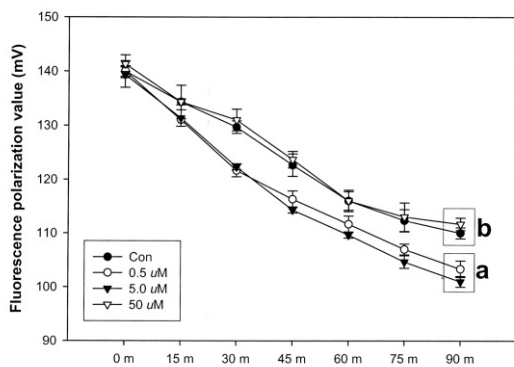


Fig. 1. Effect of iron on the caseinolytic activity of live *Uronema marinum*. The mean value of proteolytic activity  $\pm$  standard deviation (SD) are shown. The different letters denote statistically significant difference ( $P < 0.05$ ).

species, could be achieved by marking small scrape lesions on the gills and skin, and infection did not occur if the fish were not previously lesioned. Therefore, free iron in cultured water or skin of olive flounder may participate in the infection of *U. marinum* through modulation of extracellular protease activity.

### Acknowledgements

This study was supported by a grant from the Ministry of Maritime Affairs and Fisheries, Republic of Korea.

### REFERENCES

- Bolger, R. and Checovich, W.: A new protease activity assay using fluorescence polarization. *Biotechniques*, 17: 585-589, 1994.
- Cheung, P. J., Nigrelli, R. F. and Ruggieri, G. D.: Studies on the morphology of *Uronema marinum* Dujardin (Ciliata: Uronematidae) with a description of the histopathology of the infection in marine fishes. *J. Fish Dis.*, 3: 295-303, 1980.
- Dragesco, A., Dragesco, J., Coste, F., Gasc, C., Romestand, B., Raymond, J. and Bouix, G.: *Philasterides dicentrarchi*, n. sp. (Ciliophora, Scuticociliatida), a histophagous opportunistic parasite of *Dicentrarchus labrax* (Linnaeus, 1758), a reared marine fish. *Eur. J. Protistol.*, 31: 327-340, 1995.
- Dyková, I. and Figueras, A.: Histopathological changes in turbot *Scophthalmus maximus* due to a histophagous ciliate. *Dis. Aquat. Org.*, 18: 5-9, 1994.
- Gill, P. A. and Calinan, R. B.: Ulcerative dermatitis associated with *Uronema* sp. infection of farmed sand whiting *Sillago ciliata*. *Aust. Vet. J.*, 75: 357, 1997.
- Iglesias, R., Paramá, A., Alvarez, M. F., Leiro, J., Fernández, J. and Sanmartín, M. L.: *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain). *Dis. Aquat. Org.*, 46: 47-55, 2001.
- Iglesias, R., Paramá, A., Alvarez, M. F., Leiro, J., Ubeira, F. M. and Sanmartín, M. L.: *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida) expresses surface immobilization antigens that probably induce protective immune responses in turbot. *Parasitol.*, 126: 125-134, 2003.
- Jee, B. Y., Kim, Y. C. and Park, M. S.: Morphology and biology of parasite responsible for scuticociliatosis of cultured olive flounder *Paralichthys olivaceus*. *Dis. Aquat. Org.*, 47: 49-55, 2001.
- Kim, S. M., Cho, J. B., Lee, E. H., Kwon, S. R., Kim, S. K., Nam, Y. K. and Kim, K. H.: *Pseudocohnilembus persalinus* (Ciliophora: Scuticociliatida) is an additional species causing scuticociliatosis in olive flounder *Paralichthys olivaceus*. *Dis. Aquat. Org.*, 62: 239-244, 2004.
- Lall, S. P.: Nutrition and health of fish. In: Cruz - Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A., Civera-Cerecedo, R., (Eds.). *Avances en Nutrición Acuicola V. Memorias del V Simposium Internacional de Nutrición Acuicola*. 19-22 Noviembre, 2000. Mérida, Yucatán, Mexico, 2000.
- McKerrow, J. H.: Minireview: Parasite proteases. *Expl. Parasitol.*, 68: 111-115, 1989.
- Munday, B. L., O'Donoghue, P. J., Watts, M., Rough, K. and Hawkesford, T.: Fatal

- encephalitis due to the scuticociliate *Uronema nigricans* in sea-caged, southern bluefin tuna *Thunnus maccoyii*. Dis. Aquat. Org., 30: 17-25, 1997.
- Sterud, E., Hansen, M. K. and Mo, T. A.: Systemic infection with *Uronema*-like ciliates in farmed turbot, *Scophthalmus maximus* (L.). J. Fish Dis., 23: 33-37, 2000.
- Thompson, C. L. Jr. and Moewus, L.: *Miamiensis avidus* n.g.n.s., a marine facultative parasite in the ciliate order Hymenostomatida. J. Protozool., 11: 378-381, 1964.
- Yeoh-Ellerton, S. and Stacey, M. C.: Iron and 8-isoprostane levels in acute and chronic wounds. J. Invest. Dermatol., 121: 918-925, 2003.
- Yoshinaga, T. and Nakazoe, J.: Isolation and *in vitro* cultivation of an unidentified ciliate causing scuticociliatosis in Japanese flounder (*Paralichthys olivaceus*). Gyobyo Kenkyu, 28: 131-134, 1993.

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Manuscript Received : December 26, 2005

Revision Accepted : February 20, 2006

Responsible Editorial Member : Myung-Joo Oh  
(Yosu Univ.)