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☐ **Brief Communication** ☐

Supplemental knowledge on survival of *Thelohanellus kitauei* spores *in vitro*

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Abstract: In order to search the fate of *Thelohanellus kitauei* spores the extrusion rates of the polar filaments were monitored *in vitro* chronologically. Preserved spores suspended with various solutions at -70°C showed almost the same vigorous pattern as early freezing stages up to 1,750 days after initial preservation. It revealed that the viabilities of some spores suspended with 0.45% and 0.9% NaCl solutions and distilled water at 5°C continued for 1,628 days, 1,614 days and 1,721 days, respectively. And, the life spans of some spores in the previous solutions added with antibiotics at 5°C were 1,628 days, 1,614 days and 1,714 days, respectively.

Key words: *Thelohanellus kitauei*, spore, extrusion rate of polar filament, life span

Thelohanellus kitauei infection of Israel carp, *Cyprinus carpio nudus*, has been one of the most serious diseases of fish in Korea since 1988. The myxosporidian which produces giant cysts on the intestinal wall is a specific parasite of carp. In an attempt to develop the prophylactic and therapeutic measures of the disease, the course of formation and disappearance of the cysts, effects of physical and chemical factors on viability of the spores and efficacy of fumagillin dicyclohexylamine salt against the disease were reported by the author (Rhee *et al.*, 1990a & b; Rhee *et al.*, 1993).

In the previous work (Rhee *et al.*, 1990b), the life span of the spores in various suspensions was not monitored *in vitro* for long term although all the spores conserved with Tyrode's solution and the suspension added with antibiotics (penicillin 50 IU/ml and streptomycin 50 μg/ml) at 5°C were annihilated as early as 235 days after initial preservation (DAP) since a period of observation was only 760 days.

In the present study, in order to explore the fate of the spores for a period of 1,750 days, preserved spores were treated with 5% KOH solution to release the polar filament to assess survival *in vitro* after an interval of about a month (Rhee *et al.*, 1990b). Meanwhile, in the case of preservation at 5°C various suspensions were exchanged by centrifugation for new ones, whenever inspection of releasing the polar filaments (Rhee *et al.*, 1990b).

The preserved spores suspended with 0.45% and 0.9% sodium chloride solutions, distilled water and Tyrode's solution at -70°C for long term and then thawed at 5°C showed almost the same viable pattern vigorously as early freezing stages and 760 DAP (70 to 90 per cent for the extrusion rates) up to 1,750 DAP. When the preserved spores suspended with various solutions were kept at 5°C for long term, the viabilities of some spores suspended with 0.45% and 0.9% NaCl solutions and distilled water lasted for 1,628 days, 1,614 days and 1,721 days, respectively. And the life spans of some of those in the previous solutions added

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with antibiotics were 1,628 days, 1,614 days and 1,714 days, respectively.

The extrusion rates of the polar filaments of the spores were decreased with the lapse of days. There was a negative correlation between DAP and the life span of the spores in 0.45% NaCl solution. The relationship was linearly negative, and in agreement with the linear equation Y = -0.031X + 40.17, showing that the day (X) was a parameter (R = 0.7749). Similarly, it conformed with linear equations Y = -0.032X + 39.16 (R = 0.7115) in 0.9% NaCl solution and Y = -0.032X + 43.06 (R = 0.7859) in distilled water. Again, the correlations in the suspensions added with antibiotics resembled to the linear equations and it conformed with Y = -0.03X + 38.89 (R = 0.7863) in 0.45% NaCl solution, Y = -0.03X + 37.98 (R = 0.7345) in 0.9% NaCl solution and Y = -0.03X + 44.54 (R = 0.7655) in distilled water.

Ohshima (1964) reported that some of *Nosema bombycis*, a microsporidian, spores suspended with Ringer's solution at 2-5°C survived for a period of 10 years and 3 months and it was also demonstrated to maintain infectivity through the experiment. Nakajima and Egusa(1975a) indicated that the spores *of Glugea* sp., a microsporidian, in sweet fish, *Plecoglossus altivelis*, in water at 2.2-7°C survived for 105 days. Preserved spores of *Myxobolus koi*, a myxosporidian, at -10 - -20°C did not degenerate for 5 months (Nakajima and Egusa, 1974), while those of *Glugea* sp. at -20° C perished within 30 minutes after conservation (Nakajima and Egusa, 1975b).

The present experimental results are nearly coincident with the report of Ohshima (1964), although species of protozoa are different. Considering the experimental result, it is assumed that the spores maintained at -70°C will vigorously exist for many years. The preserved spores suspended with various solutions at 28°C survived for 85.4-152.4 DAP (Rhee *et al.*, 1990b), whereas those conserved at 5°C lived for a period of approximately 15-fold as long as the former. In general it is strongly suggested that the spores of myxosporidian and microsporidian protozoa should subsist for excessively long term in various low temperature environments.

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=국문초록=

*In vitro*에서 *Thelohanellus kitauei* 포자의 운명에 관한 知見補遺

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이 재 구

Thelohanellus kitauei 포자의 운명을 조사하기 위하여 *in vitro*에서 극사탈출률을 경시적으로 조사하였다. 포자를 0.45% 및 0.9%식염수, 증류수 그리고 Tyrode액에 현탁시켜 -70°C에 냉동하여 장기간 보존한 바 거의 모든 포자의 활성은 최초 냉동후 1,750일 째까지 초기와 거의 같았다. 0.45% 및 0.9%식염수 그리고 증류수에 현탁시켜 5°C에 보존한 포자는 각각 1,628일, 1,614일 그리고 1,721일, 항생제를 첨가한 위의 현탁액에서는 각각 1,628일, 1,614일 그리고 1,714일에 모든 포자가 사멸하였다. 한편, Tyrode액에 현탁시킨 것은 모두 235일에 모든 포자가 사멸하였다.

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