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Some biological characteristics of actinosporeans from the oligochaete *Branchiura sowerbyi*

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ABSTRACT: In studies on the life cycle of myxosporeans, some biological characteristics of actinosporeans (*Raabeia* sp., *Echinactinomyxon* sp., *Aurantiactinomyxon* spp., *Neoactinomyxon* sp.) from the oligochaete *Branchiura sowerbyi* kept individually in 2 ml well plates were investigated and the following results were obtained. (1) Seasonality: monthly surveys showed that prevalences of actinosporean infections reached a maximum of 1 to 5% in spring to summer and decreased in winter. Prevalences fluctuated annually during a 2 yr study period. (2) Longevity: sporoplasms of actinosporeans kept at different water temperatures (10 to 25°C) were spontaneously lost in 2 to 4 wk. Longevity differed for species and temperature: *Raabeia* sp. survived 25 d at 15°C and 10 d at 25°C. (3) Circadian rhythm of spore release: *B. sowerbyi* infected with an actinosporean were placed in 2 ml wells at 20°C under controlled lighting conditions. A significant peak of spore release was noticed at midnight and the rhythm of spore release was affected by photoperiods. (4) Chemoreception to fish mucus: when *Raabeia* sp. and *Echinactinomyxon* sp. came in contact with the skin mucus of goldfish *Carassius auratus*, common carp *Cyprinus carpio* and loach *Misgurnus anguillicaudatus*, they extruded polar filaments and released sporoplasms, however they did not react to the mucus of Japanese eel *Anguilla japonica*. It is suggested that chemoreception is related to the finding of a host by actinosporeans, and that their portal of entry is the outer surface of fish.

KEY WORDS: Actinosporean · *Branchiura sowerbi* · Myxosporean

INTRODUCTION

To date, the mode of transmission of the Myxosporea is not clearly understood; life cycle and infective stages have been demonstrated in only a limited number of species. By exposing susceptible fish to water containing unknown infectious agents or sediments from infected sites, peculiarities of infective agents have been studied (Schafer 1968, Daniels et al. 1976, Wyatt 1978, Ratliff 1983, Ching & Munday 1984, Amandi et al. 1985, Foott & Hedrick 1987, Macmillan et al. 1989). However, in studies on *Myxobolus cerebralis*, a 2-host life cycle was described (Wolf & Markiw 1984) and the infective stage to fish was the actinosporean *Triactinomyxon gyrosalmo* which infects an alternate host, the oligochaete *Tubifex tubifex*. Recent studies have suggested that actinosporeans and oligochaetes are involved in the life cycles of several other myxosporean species: *M. cotti* (see El-Matbouli & Hoffmann 1989), *M. pavlovski* (see Ruidish et al. 1991), the causative organism of proliferative gill disease (see Styer et al. 1991), *Myxobolus* sp., *Thelohanellus* sp. and *Zschokkella* sp. (Yokoyama et al. 1991), *Ceratomyxa shasta* (see Bartholomew et al. 1992), *Hoferellus carassii* (see El-Matbouli et al. 1992), *H. cyprini* (see Großheider & Körting 1992) and *M. arcticus* (see Kent et al. 1993).

In a previous paper (Yokoyama et al. 1991), a new collection method for actinosporeans was developed which made it possible to harvest pure and intact actinosporeans in large quantities. In the present study, we investigated some biological characteristics of actinosporeans using this technique and their relationship to the transmission mechanism of myxosporeans.

MATERIALS AND METHODS

Oligochaetes *Branchiura sowerbyi* Beddard from a goldfish farm in Tokyo, Japan, were kept individually

in 2 ml well plates filled with dechlorinated tap water, and waterborne actinosporeans were harvested after detection using inverted phase-contrast microscopy (Yokoyama et al. 1991). Spores released from oligochaetes were used within 24 h in the following experiments.

Seasonality. Oligochaetes at the farm were sampled every month during June 1990 to November 1991. More than 1000 *Branchiura sowerbyi* were collected each time and actinosporean infections were examined. Following the method of Janiszewska (1955, 1957), 5 species of actinosporeans were identified: *Raabeia* sp., *Aurantiactinomyxon* sp. 1, *Aurantiactinomyxon* sp. 2, *Echinactinomyxon* sp. and *Neoactinomyxon* sp. The prevalence of infection of actinosporeans was defined as the percentage of oligochaetes releasing mature actinosporeans into the water: the prevalence of infection in its strict sense (Margolis et al. 1982) could not be determined, because the immature stages infecting the oligochaete were not counted by this method.

Longevity. Incidental observations showed that sporoplasms were spontaneously lost and only spore valves remained several weeks after release into the water. Spores with or without sporoplasms were easily distinguished under phase-contrast microscopy at 100× magnification, and spores with sporoplasm were defined as viable. Actinosporeans of the genera *Raabeia*, *Aurantiactinomyxon* or *Echinactinomyxon* were suspended in dechlorinated tap water at a concentration of 7000 spores ml^{-1} in 10 ml sterilized plastic tubes. Each tube was then placed at a constant temperature of 10, 15, 20 or 25°C. Viability of 100 spores was examined daily.

Circadian rhythm of spore release. *Branchiura sowerbyi* infected with an actinosporean were put individually in 2 ml wells at a constant temperature of 20°C. They were placed 25 cm away from a 15 W incandescent light bulb suspended in the incubator. In order to illuminate from above only, the bottom was covered with black paper. The oligochaetes at the farm were collected in the morning, packed in a transparent vinyl bag and transferred to our laboratory by noon. *B. sowerbyi* were individually selected into well plates from 13:00 to 18:00 h under normal lighting conditions. The plates were left overnight in a 20°C incubator with the photoperiod of 18:00 to 6:00 h dark and 6:00 to 18:00 h light. The day after, actinosporean infections were examined, then 10 *B. sowerbyi* infected with *Echinactinomyxon* sp. were selected and divided into 4 groups: (1) normal photoperiod of 6:00 to 18:00 h light, 18:00 to 6:00 h dark; (2) reversed photoperiod of 6:00 to 18:00 h dark, 18:00 to 6:00 h light; (3) constant light; and (4) constant dark. At 4 h intervals, the number of spores in a 100 µl aliquot was counted and subsequently *B. sowerbyi* were individually transferred to a new well. This experiment was performed on 22 to 28 July 1991.

Chemoreception to fish mucus. Skin mucus from healthy fish (goldfish *Carassius auratus*, common carp *Cyprinus carpio*, loach *Misgurnus anguillicaudatus* and Japanese eel *Anguilla japonica*) was collected by cotton moistened with distilled water, and smeared onto a clean glass slide. Actinosporean suspensions were added to the mucus and examined by phase-contrast microscopy. Intensity of reaction was determined by counting the number of empty spores without sporoplasms from 100 spores examined.

RESULTS

Seasonality

During the study period, significant seasonal fluctuations were found in prevalences of the 5 species of actinosporeans (Fig. 1). The prevalences were 1 to 5% at most in spring to summer (May to October) and very low in winter. There was almost a single peak in the year, the highest prevalence in each year was 4.2% of *Aurantiactinomyxon* sp. 1 in June 1990, and 4.4% of *Echinactinomyxon* sp. in June 1991. The pattern of *Neoactinomyxon* infection differed from the other species; it was very low (0 to 0.1%) during June to September but increased to 1.0% in October 1990. Annual fluctuations as well as seasonal changes were noticed; species found frequently in 1990, *Aurantiactinomyxon* sp. 1 (at max. 4.2%), *Aurantiactinomyxon* sp. 2 (at max. 2.5%) and *Raabeia* sp. (at max. 2.3%), decreased to less than 1% in the following year. On the other hand, the prevalence of *Echinactinomyxon* sp. was less than 1% in 1990 and increased to a maximum of 4.4% in 1991.

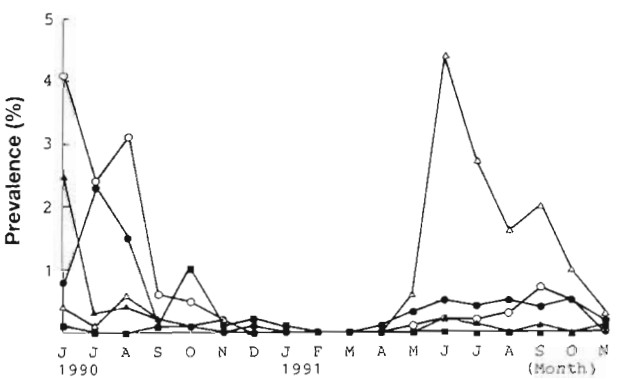

Fig. 1 Seasonality of prevalences of actinosporean infections in the oligochaete *Branchiura sowerbyi* during June 1990 to November 1991. (●) *Raabeia* sp.; (○) *Aurantiactinomyxon* sp. 1; (▲) *Aurantiactinomyxon* sp. 2; (△) *Echinactinomyxon* sp.; (■) *Neoactinomyxon* sp.

Longevity

Longevity decreased with increasing temperatures and differed among species: *Raabeia* sp. survived 25 d at 15°C and 10 d at 25°C, while the viability of *Aurantiactinomyxon* sp. 1 persisted for 11 d at 15°C and 4 d at 25°C, that of *Echinactinomyxon* sp. was more than 25 d at 15°C and 14 d at 25°C (Fig. 2).

Circadian rhythm of spore release

On the first day of the experiment, the peak of spore release was observed at midnight (22:00 to 2:00 h) in all groups and then almost a 24 h cycle was repeated in normal photoperiods. In reversed photoperiods, the release pattern was also reversed. In total light and total darkness, no clear circadian rhythm was recorded (Fig. 3).

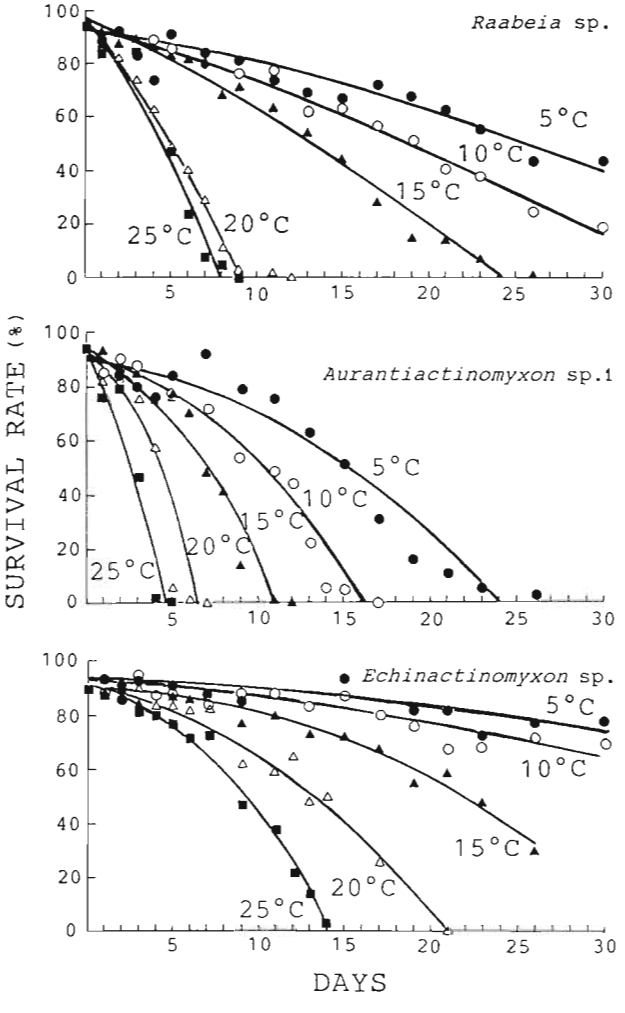

Fig. 2. Longevity of actinosporeans kept at different temperatures

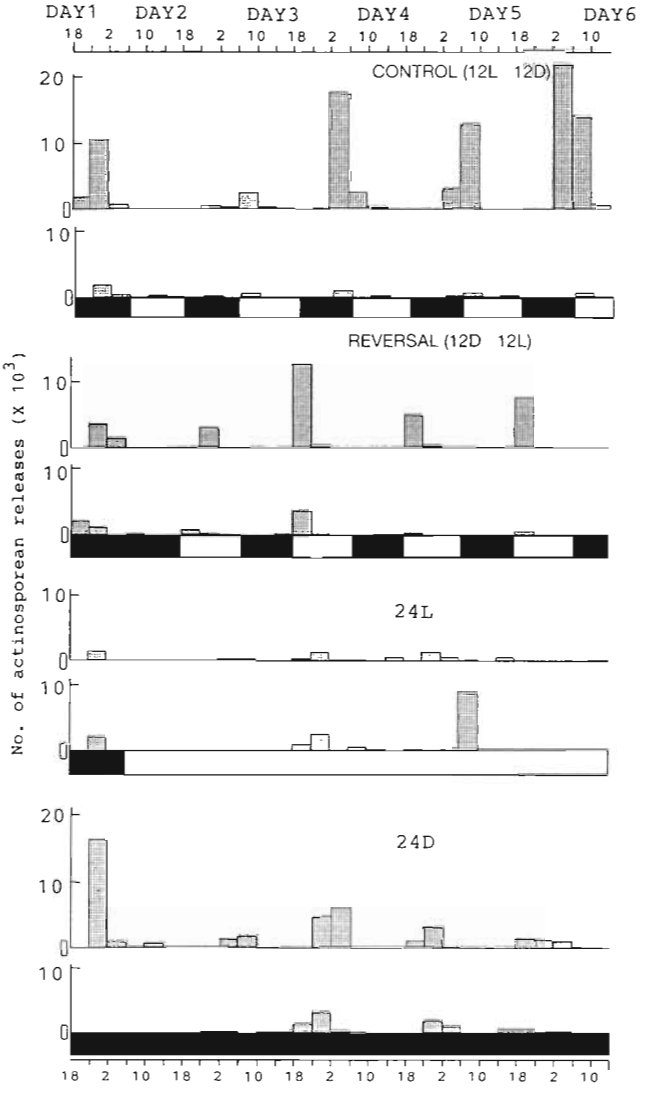

Fig. 3. Circadian rhythm of spore release of *Echinactinomyxon* sp. from 8 individuals of *Branchiura sowerbyi* kept in 2 ml well plates under controlled photoperiods. Black and white columns show the dark and light hours, respectively

Chemoreception to fish mucus

Instantaneous responses of sporoplasm release occurred in *Raabeia* sp. and *Echinactinomyxon* sp. which came in contact with the mucus of goldfish, carp and loach. Polar filaments were extruded and discharge of polar filaments out of the epispore was accompanied by the release of the sporoplasm outside (Fig. 4). However, they did not react with the mucus from Japanese eel. Intensities differed among fish species; 46 to 66% of *Raabeia* sp. spores contacted with goldfish mucus released sporoplasms, while 73 to 80% reacted with common carp mucus and 72 to 78% with loach mucus (Table 1). It is possible, though, that the

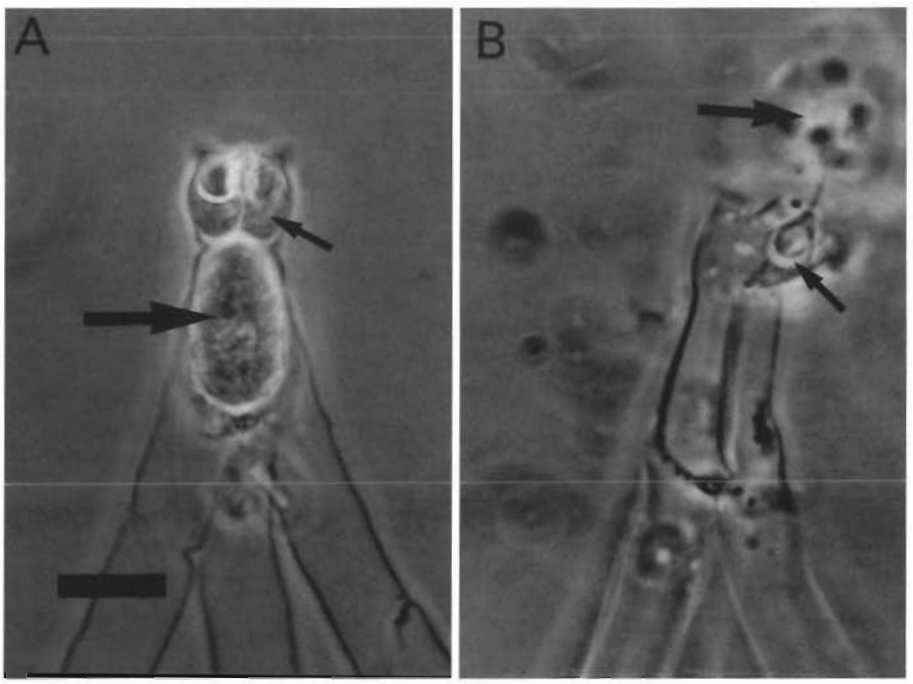

Fig. 4. *Raabeia* sp. Sporoplasm release when in contact with fish mucus. (A) Intact spore. (B) Spore after contact with goldfish mucus. Large and small arrows show sporoplasms and polar capsules, respectively. Scale bar = 10 µm

variability of the data may have resulted from the differences in the quantity of mucus smeared on the slide. *Aurantiactinomyxon* sp. 1 did not react with the mucus from any of the 4 fish species used.

DISCUSSION

Results of this study on the characteristics of actinosporeans give some new insights on the transmission mechanism of myxosporeans to fish, although at present transformations of these actinosporeans into myxosporeans have been experimentally demonstrated in only 1 species, *Raabeia* sp., which transformed into *Myxobolus* sp. in goldfish (Yokoyama et al. 1991).

In the monthly surveys of oligochaetes, seasonal appearance of actinosporeans directly indicates infective periods to fish. Several myxosporeans show seasonal and annual patterns of infection caused by endogenous cycles of the parasites, availability of susceptible hosts or the effects of environmental factors (Foott & Hedrick 1987). In this study, it was shown that abundance of actinosporeans may also be a factor affecting seasonality.

Concerning longevity of actinosporeans, actinosporean infectivity remains to be studied. Ratliff

Table 1 Chemoreception of actinosporeans to mucus from different species of fish; sporoplasms are released immediately after contact with mucus. Numbers show percentage of empty spores without sporoplasms (n = 100). *Significantly different from control by chi-square analysis; –: not determined

Actinosporean	Control	Mucus from different fish species			
		Goldfish *Carassius auratus*	Common carp *Cyprinus carpio*	Loach *Misgurnus anguillicaudatus*	Japanese eel *Anguilla japonica*
Raabeia sp.	8	74*	88*	86*	–
	7	51*	70*	79*	13
	2	48*	75*	–	6
Aurantiactinomyxon sp.	6	8	4	1	–
	19	11	19	15	10
	16	24	14	–	18
Echinactinomyxon sp.	1	89*	53*	94*	–
	4	88*	93*	98*	11
	4	70*	87*	–	4

(1983) showed that the water containing some unknown infective agent of *Ceratomyxa shasta* kept its infectivity to fish for 7 d. Recently Markiw (1992) demonstrated that the viability of the actinosporean stage of *Myxobolus cerebralis* persisted 5 d at 12.5°C by *in vitro* vital staining with fluorescein diacetate and propidium iodide, and the infectivity of the stage at 12.5°C was kept for 3 d by *in vivo* exposure of fish. Longevity, defined in the present study as the period from the release of spore into the water until the sporoplasms were lost, means the time to total disintegration of actinosporeans. Therefore, the infectivity to fish may be overestimated if assumed to be comparable to longevity in the true sense of the term.

The presence of a release rhythm of actinosporeans from the oligochaete is reported for the first time in this study and it is not clear whether this phenomenon has any special meaning in infection to fish. The rhythmic emergence of a parasite from an intermediate host or egg has been described by several authors: the trematode *Schistosoma mansoni* (see Asch 1972), the monogenean *Entobdella soleae* (see Kearn 1973) and the trematode *Halipegus occidualis* (see Shostak & Esch 1990). This phenomenon has been considered to have an ecological significance in transmission to the next host; some hypotheses proposed are synchronization with activity of the host, enhancement of dispersal and reduction of mortality (Shostak & Esch 1990). However, actinosporeans apparently do not possess a photosensory organ, so that the daily rhythm found in this study seems more likely to be the rhythm of the oligochaete itself than that of the actinosporean. As far as is known, light-dependent circadian clocks in oligochaetes have not been demonstrated. Oligochaetes may have a kind of physiological rhythm, probably a feeding rhythm, because actinosporeans are thought to be released from the alimentary tract with faeces.

The actinosporean responses to fish mucus in this study suggest that chemoreception is present in the host-finding mechanism of actinosporeans, and that their portal of entry is the outer surface of fish. Furthermore, it is possible that polar capsules have a role as a 'cap' to hold sporoplasms. In the event of an infection just after host recognition by chemoreception, polar capsules are removed so that sporoplasms can exit from the top of the epispore. *In vivo* infection experiments of goldfish to *Raabeia* sp. have revealed that 80 to 98% of the *Raabeia* sp. released sporoplasms within 2 to 3 h (H. Yokoyama, K. Ogawa & H. Wakabayashi unpubl). Markiw (1989) also observed the release of sporoplasms when rainbow trout were exposed to the actinosporean stage of *Myxobolus cerebralis*.

Results of the present study raised further questions essentially related to host specificity and transmission mechanism of myxosporeans. These include: is there a species-specific substance (or substances) in the fish mucus? Does *Raabeia* sp. invade other species of fish and develop normally? Why did the mucus from Japanese eel not activate the sporoplasm release of actinosporeans? Why did *Aurantiactinomyxon* sp. 1 not react with mucus? Further biological and chemical studies are required to elucidate these uncertainties.

In conclusion, the study of some biological characteristics of actinosporeans showed the peculiarity of myxosporean infections to fish; *Raabeia* sp. in the oligochaete was released to water at midnight during spring to summer. While floating in water with the help of the long appendages for at most 10 d, they recognize fish by certain chemical substances in fish mucus, and then extrude polar filaments, which makes it possible for sporoplasms to invade the fish.

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