# Polar filament discharge of *Myxobolus cerebralis* actinospores is triggered by combined non-specific mechanical and chemical cues

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SUMMARY

This study presents initial evidence for the requirement of both chemical and mechanical stimuli to discharge polar capsules of *Myxobolus cerebralis* actinospores, the causative agent of salmonid whirling disease. The obligate need for combined discharge triggers was concluded from data obtained in a before/after experimental set-up carried out with individual locally immobilized actinospores. Homogenized rainbow trout mucus as chemostimulus and tangency of the apical region of the spores to achieve mechanical stimulation were applied subsequently. The actinospores showed discharged polar filaments exclusively when mucus substrate application was followed by touching the polar capsule-bearing region, but not when either stimulus was offered solely to the same individuals. We measured filament discharge rates to mucus preparations in a microscopic assay using supplementary vibration stimuli to ensure mechanical excitation. The actinospores responded similarly to different frequencies, which suggested a touch-sensitive recognition mechanism. Discharge specificity for salmonid mucus could not be confirmed, as mucus of common carp and bream could trigger similar filament expulsion rates. To a lesser extent homogenized frog epidermis and bovine submaxillary mucin could also stimulate the attachment reaction. In contrast, mucus of a pulmonate freshwater snail elicited no response.

Key words: Myxozoa, *Myxobolus cerebralis*, polar capsule discharge, chemo-perception, host specificity, mucus.

## INTRODUCTION

Whirling disease, caused by the cosmopolitan parasite *Myxobolus cerebralis*, is still considered to be one of the most devastating diseases among rainbow trout populations (Hoffman, 1990; Gilbert and Granath, 2003). It has been responsible for huge declines of the wild rainbow trout population in more than 22 US states (Hedrick *et al*. 1998). The passively floating actinospores play a key role in the spread of the infective sporozoites, but myxozoan actinospores do not remain infective for as long as myxospores, which are able to retain their infectivity for more than 20 years (El-Matbouli, Fischer-Scherl and Hoffmann, 1992). Waterborne *M. cerebralis* triactinomyxon-type actinospores have to invade their host in less than 60 h after release (Markiw, 1992), and thus would benefit from functional adaptations for adequate host recognition. Host attachment is mediated by the extrusion of an eversible tubule from apically located polar capsules reminiscent of cnidarian nematocysts (Lom, 1990; Siddall *et al*. 1995). Though recent findings revealed strong evidence for triploblast bilateral ancestors of the myxozoan phylum (Schlegel *et al*. 1996; Anderson, Okamura and Canning, 1998; Okamura *et al*. 2002), there are arguments for a cnidarian origin of the polar capsules (discussed by Canning and Okamura, 2004; see also Kent *et al*. 2001; Siddall *et al*. 1995). The polar filaments were compared to the stinging threads of the parasitic cnidarian *Polypodium hydriforme* by Ibragimov (2001), and discharge reactions to various chemicals and structural aspects were summarized by Cannon and Wagner (2003).

The polar filament discharge reaction takes place rapidly and depends upon coincidental contact, e.g. by breathing or fin movement of the fish host. Following attachment, the infective amoeboid sporoplasm actively penetrates the host integument through the opening of mucous cells in the skin (El-Matbouli *et al*. 1999). The physiological mechanisms and cues that underlie these reactions have long been a matter of speculation and remain unknown (Kent *et al*. 2001). Although some researchers managed to show reactivity of actinospores to fish mucus (Yokoyama, Ogawa and Wakabayashi, 1993, 1995; Uspenskaya, 1995; McGeorge, Sommerville and Wootten, 1997; Xiao and Desser, 2000; Ozer and Wootten, 2002), *M. cerebralis* actinospores could not be stimulated significantly to discharge filaments or release their sporoplasms when brought in

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contact with salmonid mucus or epidermal tissue (El-Matbouli *et al*. 1999; Wagner, 2001 *a*). A random screening of chemicals known to discharge nematocysts in order to find host-related substances involved in the discharge reaction of actinospores was equally unsuccessful (Wagner, 2001 *b*).

It is long known that in whirling disease the development of mature spores in the teleost host is restricted to salmonids, although the question by which means this specificity is generated is unanswered. For *M. cerebralis* actinospores, it would be of great benefit to avoid interference by incompatible hosts and other aquatic organisms encountered. There is some indication of host specificity in the very first steps of host invasion for *M. cerebralis*, based on the absence of parasite stages in histological examinations and unsuccessful infection in various experimentally exposed non-salmonids (El-Matbouli *et al*. 1999).

In the present study, we elucidated host cues that trigger polar filament discharge and examined the possibility of a host-specific attachment reaction by *M. cerebralis* actinospores. We hereafter demonstrate a dependence on mechanical and mucus-derived cues. Tests using mucus from various animals were applied to evaluate the degree of host specificity during the attachment reaction.

## MATERIALS AND METHODS

### *Parasites*

*Myxobolus cerebralis* and its oligochaete host *Tubifex tubifex* originated from the Institute of Zoology, Fish Biology and Fish Diseases of the University Munich, Germany. Infected oligochaete cultures were kept at 15 °C in the dark and were fed on a mixture of freeze-dried *Spirulina*, Algamac 2000® (Aquafauna Bio-Marine Inc., Hawthorne, CA), frozen artemias and minced frozen lettuce weekly. The actinospores were concentrated from the oligochaete cultures by passing the water through 20 $\mu$m nylon meshes. To assess the physiological condition and infectivity, the viability status of the cellular components of the routinely harvested actinospores up to 24 h of age was recorded by fluorescein-diacetate/propidium iodide (FDA/PI) double staining as described by Yokoyama *et al*. (1997) and Markiw (1992) from spore isolates in 3 different weeks.

### *Test substrates*

Adult rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) were obtained from a local distributor. Common bream (*Abramis brama*) were caught in the river Aisch, Germany. Fish were killed by a sharp blow to the head and mucus was scraped off with a blunt knife while rubbing small amounts of deionized water onto the fish surface. The collected mucus was homogenized using an Ultra Turrax (IKA Labortechnik, Staufen) and suspended by sonication, followed by centrifugation to remove insoluble components. Mucus of *Lymnaea stagnalis* was collected by scraping the walls of a small PVC container holding 50 large snails in 15 ml of water, the remaining faeces being removed by centrifugation. The mucus was concentrated by removing water from the solution by partial lyophilization. Frog epidermal extract was obtained by pulverizing stratum corneum patches of freshly killed *Rana esculenta* specimens by addition of liquid nitrogen, then homogenizing after addition of 0·4 times the wet weight of deionized water. Centrifugation at 2200 ***g*** and 4 °C for 10 min yielded a fully soluble supernatant. The supernatants, hereinafter referred to as 'mucus', were stored at −75 °C.

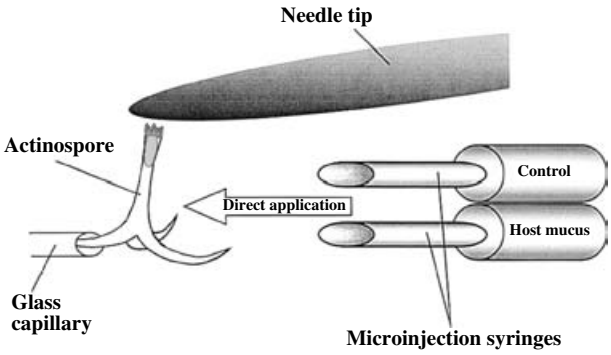


Fig. 1. Schematical illustration of the experimental setup to evaluate the dependency between mechanical stimulation and trout mucus application to trigger polar capsule discharge of individual *Myxobolus cerebralis* actinospores.

Trout muscle tissue homogenate was prepared from muscle sections from fresh filets (5 g/3 ml deionized water), then the concentrated muscle tissue solutions (5 mg/ml final concentration) were desalted by molecular filtration at 4 °C using Centricon concentrators (Amicon) with cut-off at 3 kDa. The retentates were washed with half the original filling volume of deionized water to remove low molecular weight remnants. To adjust mucus isolates to the same carbohydrate content, neutral sugar concentration was measured by the Resorcinol/$H_2SO_4$-method described by Monsigny, Petit and Roche (1988) in 96-well microtitre plates, mannose served as standard.

### *Quantification of polar capsule discharge*

By observing the reactions of single actinospores, the influence of mechanical stimulation on polar filament discharge was examined before and after subsequent treatment under a stereomicroscope. The spores were immobilized by sucking in one caudal process into the opening of glass capillaries on a moulded slide (Fig. 1). Test and control substrates were applied directly covering the whole spore by

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2 separate Hamilton glass syringes (10 $\mu$l volume). All elements were movable by micromanipulators. The mechanostimulus was administered by gently touching the apex 3 times at its polar capsule bearing apical ending with the tip of a stainless steel needle (0·5 mm diameter). Each actinospore received a mechanical stimulus after application of 1·5 $\mu$l control (buffered deionized water). Then 1·5 $\mu$l of crude trout mucus were added, followed by another mechanostimulus. After each stimulation step (chemical and mechanical), the actinospores were checked for extruded filaments for 10 s and were considered discharged if, at that time, 1 of the 3 filaments was extruded. In a control trial, we offered control water as second chemostimulus prior to the second mechanostimulation to exclude an effect of repeated stimulation. The experiment was conducted with actinospore isolates from 10 different days.

Bulk examination of polar filament extrusion rates (vibration microassay) was performed on microscope glass slides. An amount of 9 $\mu$l of buffered test substrate was added to 21 $\mu$l of buffered spore suspension on slides and covered with a cover-slip (20 × 20 mm). To provide the mechanical stimulus, the preparation was placed on a vibration apparatus (Mini Shaker Type 4810, Brüel & Kjær, Copenhagen), which was driven by an amplified laboratory frequency generator (FG 200, HTronic GmbH, Hirschau). The slide was vertically shaken for 3 s at 50 Hz (amplitude 0·5 mm), which was permanently controlled by an oscilloscope (Tektronix, Cologne) with triggered differential amplification. Responses to different frequencies were examined the same way, whereas 4 $\mu$l of trout mucus (1 mg/ml) were added to 16 $\mu$l of spore suspension to stabilize the system at higher frequencies. The frequency and amplitude settings were altered to calibrated values to give the same vertical deflection amplitude. Buffered deionised water served as control in all experiments.

Spores were considered discharged if at least 1 polar filament could be seen extruded. All experiments were conducted at room temperature under a binocular microscope using magnifications of × 200–400. Only undamaged, viable spores that were not older than 48 h were included in the evaluation. All media, containing parasites or test substrates were adjusted to pH 7·5 (5 mM sodium phosphate buffer). The spore suspension was stored at 15 °C and the test substrates were kept on ice during use. Except for the paired sample test procedure and the frequency response test, a blinded protocol was used by allocating the test substrates codes unknown to the investigators.

### *Statistical methods*

Statistical analysis was performed using SPSS for Windows. Relative abundances were Arcsine square root transformed to obtain approximately normally distributed data. Mean values and standard errors were computed from the transformed data and were retransformed for graphical visualization. For simplification, only the larger of the two different standard errors is indicated in the table. Differences between means were tested for statistical significance by a multivariate comparison procedure (Tukey HSD multiple t-test after One-Way-ANOVA and the Levene-test for homogeneity). Differences in change of response of the nonparametric data that resulted from the paired samples test of individual spores were determined by the McNemar $\chi^2$-procedure for nominal dichotomous variables in before/after designs.

## RESULTS

FDA/PI double-staining showed that an average of 48·5% (± 3·3) were fully viable on the cellular level of the spores that were expelled less than 24 h before filtration. Single discharged polar capsules accounted for more than 90% of the not fully viable individuals (202 spores counted, 3 replicates). In virtually all actinospores the infective germs including the sporoplasm cells were viable, about 5% showed dead amoeboid germ cells along with considerable damage of the spore valves.

The subsequent exposure of 47 individual spores to host mucus and mechanical stimulation revealed the need for sensitizing chemostimuli followed by a touch signal to elicit polar filament discharge. In a first step, water was applied to the actinospores, which caused no discharge of polar filaments. Thereafter, the same actinospores received the mechanical stimulation, where 1 of the 47 actinospores reacted. The ensuing mucus application alone did not cause discharge in any specimen. Among hitherto non-extruded spores that were fully covered in trout mucus at this step, 54% of the tested individuals extruded one or more filaments only after further mechanostimulation (25 spores, significant change of response *vs* subsequent control/mechanostimulus/trout mucus-application, McNemar $\chi^2$-procedure ($P < 0{\cdot}001$)). In the control trial, water replacing trout mucus prior to the second mechanostimulus did not cause discharge of the polar capsules as only 2 individuals reacted after the first mechanostimulus and none after the second mechanostimulus (17 spores, $P < 0{\cdot}001$ *vs* the same step in the mucus trial, McNemar $\chi^2$-procedure).

When actinospore discharge rates were measured using the vibration-microassay (bulk experiments), a test proved that there was no difference in discharge rates between buffered deionized water and buffered and non-buffered water from the spore filtrate (data not shown). A frequency-dependent discharge reaction was not observed, but trout mucus raised the reaction rates by about 20% over the whole frequency range (Fig. 2; $P < 0{\cdot}05$ with mucus *vs* control at all

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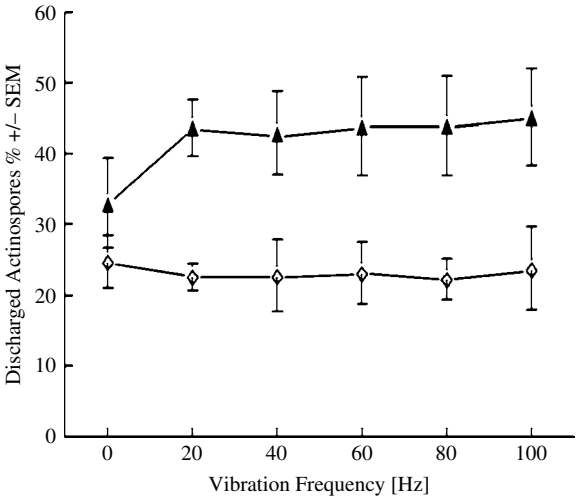


Fig. 2. Effect of vibration frequency on discharge rates (%) by *Myxobolus cerebralis* actinospores after exposure to trout mucus (solid triangles) and water (open triangles); 285–493 actinospores counted per frequency, 7 replicates.

frequencies). Interestingly, an increased discharge rate could already be noted when no vibrations, but mucus was applied (discharge rate at 0 Hz, Fig. 2).

Trout mucus was an effective discharge trigger at various concentrations (Table 1 (A)). Carp mucus triggered polar capsule discharge as effectively as trout mucus (Table 1 (B); $P=0{\cdot}74$ *vs* trout mucus). Bream mucus was similarly effective ($P=0{\cdot}63$ *vs* trout mucus at the same concentration), though it did not reach the same discharge percentage as trout mucus, even at higher concentrations (Table 1 (C)). Freshwater snail mucus seemed to lack the required triggers (Table 1 (D)). *Lymnaea* mucus neither stimulated in the same dry weight concentration as trout mucus ($P<0{\cdot}001$ *vs* trout mucus), nor when adjusted to the same carbohydrate content as trout mucus (Table 1 (E); $P=0{\cdot}002$) or even higher concentration (Table 1 (E)). Frog epidermal substrate (*Rana* epidermis) could trigger discharge by increasing the dry weight concentration (Table 1 (F) and (G); 5 mg/ml, $P=0{\cdot}94$ *vs* trout mucus and 0·001 *vs* control). Bovine submaxillary mucin was a weaker chemostimulant (2 mg/ml, $P=0{\cdot}25$ *vs* control, 1·00 *vs*.frog (1 mg/ml), 0·19 *vs* frog (5 mg/ml) and 0·03 *vs* trout mucus) and increased concentrations led to even fewer responses (Table 1 (G); 5 mg/ml, $P=0{\cdot}678$ *vs* control, 0·038 *vs* frog (5 mg/ml)). To designate the recognized signal as mucus-specific, homogenized trout muscle tissue was offered. This substrate caused relatively low discharge rates even at very high concentrations (Table 1 (H); 5 mg/ml, $P=0{\cdot}32$ *vs* trout mucus).

## DISCUSSION

Parasite invasion can be differentiated as a sequence of responses to certain stimuli as seen in trematode

Table 1. Polar capsule discharge of *Myxobolus cerebralis* actinospores in response to different substrates mixed on a slide with application of vibrations (3 s, 50 Hz, Amplitude 0·5 mm)

| Substrate | Discharge (% ± S.E.M.) |
|---|---|
| (A) Host fish mucus (*Oncorhynchus mykiss*) | |
| Trout mucus 0·1 mg/ml | 53·3 ± 12·4 |
| Trout mucus 1·0 mg/ml | 75·0 ± 4·7** |
| Trout mucus 5·0 mg/ml | 76·8 ± 3·9** |
| Control | 27·8 ± 7·4 |
| (B) Non-host fish mucus (*Cyprinus carpio*) | |
| Trout mucus 1 mg/ml | 51·7 ± 6·7** |
| Carp mucus 1 mg/ml | 46·8 ± 6·6** |
| Control | 20·3 ± 3·6 |
| (C) Non-host fish mucus (*Abramis brama*) | |
| Trout mucus 1 mg/ml | 61·2 ± 9·8* |
| Bream mucus 1 mg/ml | 48·0 ± 11·4 |
| Bream mucus 2 mg/ml | 51·0 ± 9·2 |
| Bream mucus 3 mg/ml | 55·7 ± 8·6 |
| Control | 27·2 ± 6·8 |
| (D) Snail mucus (*Lymnaea stagnalis*) | |
| Trout mucus 1 mg/ml | 51·0 ± 7·7** |
| Snail mucus 1 mg/ml | 13·8 ± 2·6 |
| Control | 11·4 ± 1·7 |
| (E) Snail mucus (concentrated) | |
| Trout mucus 1 mg/ml | 53·2 ± 4·7** |
| Snail mucus 1·56 mg/ml[a] | 23·0 ± 4·4 |
| Snail mucus 5 mg/ml | 30·8 ± 6·3 |
| Control | 23·0 ± 3·2 |
| (F) Frog epidermis (*Rana esculenta*) | |
| Trout mucus 1 mg/ml | 65·7 ± 7·8** |
| Frog skin homogenate 1 mg/ml | 39·0 ± 5·8 |
| Frog skin homogenate 5 mg/ml | 59·2 ± 6·3** |
| Control | 25·1 ± 3·6 |
| (G) Bovine submaxillary mucin | |
| Trout mucus 1 mg/ml | 65·7 ± 7·8** |
| Bovine submaxillary mucin 2 mg/ml | 41·1 ± 6·5 |
| Bovine submaxillary mucin 5 mg/ml | 35·6 ± 6·3 |
| Control | 25·1 ± 3·6 |
| (H) Non-mucus host tissue | |
| Trout mucus 1 mg/ml | 61·2 ± 6·6* |
| Trout muscle 1 mg/ml | 32·0 ± 3·9 |
| Trout muscle 5 mg/ml[b] | 39·0 ± 8·9 |
| Control | 31·5 ± 7·8 |

* $P \leqslant 0{\cdot}03$ *vs* control ** $P \leqslant 0{\cdot}001$ *vs* control (Tukey HSD t-test).

[a] Adjusted to the same total carbohydrate content as trout mucus.

[b] Desalted by molecular filtration (cut-off 3 kDa)

Note: Total number of actinospores counted per substrate (A) 80-151 (five replicates), (B) 216-355 (seven replicates), (C) 713-1179 (six replicates), (D) 290-481 (six replicates), (E) 209-337 (twelve replicates) (F) 515-674 (six replicates), (G) 657-733 (six replicates), (H) 546-928 (eight replicates).

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cercariae (reviewed by Haas and Haberl, 1997; Haas, 2003). Host attachment, penetration by actinospores and migration of the amoeboid germ should be considered as separate steps, possibly requiring different cues. Our data indicate that *M. cerebralis* actinospores require a chemical, mucus-derived signal before they become mechanically sensitive for polar filament discharge. The combination of both triggers, formerly suggested by El-Matbouli *et al.* (1999), makes sense, as the length of the polar filament (about 30–40 $\mu$m) can mediate host surface anchorage only when close contact occurs. A discharge reaction caused by chemical or mechanical signals individually would most certainly lead to inefficient spontaneous discharge. Examples of a dual need for chemical and mechanical stimuli are numerous in coelenterates, but the actual chemoreceptors and their location have rarely been described (Kass-Simon and Hufnagel, 1992).

The maximum percentages of reacting actinospores varied from about 40 to nearly 80% in the bulk experiments. We suggest that this variability is caused by differences in spore age as shedding of the actinospore packets by oligochaetes cannot be triggered and therefore all experiments include spores of different age. For example, chemoreceptor function could be affected by spore age. It is not known whether non-viable spores may contribute to the discharge rates measured in our experiments. Actinospores with non-viable polar capsule cells could lower the measured discharge rate. Markiw (1992) observed dead sporoplasm cells and intact polar capsules in the same spores when using the FDA/PI staining method. Thus, discharging spores with dead sporoplasms can give a wrong impression if the discharge rate is put on a level with infectivity. The fact that the spores showed intact amoeboid germs and very few were impaired on a cellular level except for single discharged polar capsule cells renders this factor negligible. When the results from our control substrates are compared with the measured cellular viability rate, it becomes clear that the staining procedure gives a wrong picture on polar capsule cell viability by causing discharge in several spores. Therefore, the actinospore isolates used in our tests were suitable for our investigations.

Lowered osmolarity alone was not responsible for the low discharge rates with deionized water as control, as buffered spore medium water could not trigger discharge. However, osmolarity gradients might be involved in the discharge reaction (see also Yokoyama *et al.* 1995). The attempts to increase discharge rates by using higher concentrated mucus homogenates show the existence of a threshold concentration in our experiment at about 1 mg/ml. Of course, when encountering the fish host, actinospores encounter a much denser (highly concentrated) mucus layer. Our results also show that the discharge reaction is independent of physical properties and the pH of the living fish surface.

The question why several myxozoan species show a narrow invertebrate host range but are able to successfully develop in a rather wide choice of teleost host species (e.g. salmonids for *M. cerebralis*) is not yet understood. It was postulated, that actinosporeans specifically recognize their respective hosts. Xiao and Desser (2000) observed differences in sporoplasm release ratio by an array of actinospores to mucus of various fish. Also, *Thelohanellus hovorkai* was reported to distinguish between cyprinid genera (Yokoyama *et al.* 1997). These authors concluded that the spores did not penetrate non-susceptible fish by the absence of myxospore production and from the fact that no parasite stages were found in tissues. Other observations by the latter authors revealed penetrated cells, but no reproduction of *M. arcticus* in non-susceptible salmonids. Going even further, Hoffmann, El-Matbouli and Hoffmann-Fezer (unpublished observations) reported that *M. cerebralis* actinospores were able to distinguish naïve from infected host fish by a lack of filament discharge when exposed to the latter. According to our opinion, differences in threshold concentrations triggering the actinospore's receptors, varying moieties of cues present in mucus of different fish species and different mechanical sensitivity could be reasons for such varying discharge rates.

On the other hand, Yokoyama *et al.* (1995) found raabeia-type actinospores of *M. cultus* frequently reacting to various fish mucus isolates as well as to bovine submaxillary mucin. McGeorge *et al.* (1997) and Ozer and Wootten (2002) exposed several actinosporeans to mucus of salmon, trout, stickleback and bream and found all of them reacting to all mucus isolates by polar filament discharge and sporoplasm hatching. Although these authors did not apply a separate mechanostimulus except agitation and cover-slips, these findings support our results on the discharge triggering activity of mucus isolates from host fish species and non-susceptible fish.

The triggering activity of carp and bream mucus at similar concentrations demonstrated, at the level of attachment, the failure of *M. cerebralis* actinospores to discern host fish species from species in which the parasite is unable to develop. This is underlined by the fact that the amoeboid germs were actively leaving the spore in non-host mucus (unpublished data). It is therefore suggested, that unsusceptible fish species might serve as potent decoy organisms in waters enzootic for *M. cerebralis* due to developmental failure after penetration. This is in contrast to the results obtained by El-Matbouli *et al.* (1999) who did not observe a prospected amount of attached *M. cerebralis* actinospores on exposed carp. Our data must be interpreted carefully, since an artificial stimulation of actinospores does in no way represent

the true biophysical situation at the surface of a living fish. Furthermore, it is not certain that the same compounds in carp and trout mucus caused discharge.

The low responses to trout muscle homogenate indicate that a mucus-specific compound is recognized by actinospores. An attachment preference towards mucous cell openings as site of entry gives an additional hint to link the discharge signal to the products of granular glands (El-Matbouli *et al*. 1999). Muci of other vertebrates such as *Rana* epidermal homogenate triggered significant discharge rates. Whole frog stratum corneum patches contain great amounts of granular goblet- and Leydig-cell products, and therefore can serve as a comparable mucous substrate. As some myxozoan species are known to parasitize anuran hosts (reviewed by Browne *et al*. 2002), this result was not surprising. Together with the reactions to bovine submaxillary mucin, this alludes to a signal that is common in vertebrate mucus. Bovine mucin was found to be a potent trigger for cnidae discharge in anemones as well (Watson and Hessinger, 1987). Nevertheless, our results with bovine mucin remain ambiguous, as it did not show any triggering effect at 1 mg/ml (data not shown), and responses varied greatly when the concentration was increased.

During transmission, it is imperative that actinosporean stages avoid accidental discharge at contact, not only with dead organic matter, but also with aquatic invertebrate surfaces and secretions (most notably of their own oligochaete hosts) and other plankton organisms such as crustaceans and protozoans. Considering transmission stages of other parasites that invade freshwater teleost hosts, to date our knowledge is limited concerning host cues and the specificity of their reactions and behavioural patterns. One example for high species-specific reactions in fish parasites is the oncomiracidium of *Entobdella soleae* (Kearn, 1967). *Diplostomum spathaceum* cercariae, actively invading a wide range of host fish, also rely on host contact by chance. This species uses the increased $CO_2$ pressure for their attachment response (Haas, 1975), which is a very unspecific signal. The cercariae readily attach to various aquatic animals, but penetration only takes place, when specific macromolecular substances (sialic acid containing glycoproteins) and lipids are present (Haas *et al*. 2002). Frog mucus, producing high amounts of bicarbonate (Dapson, 1970), would also provide a high $CO_2$ pressure that could be utilized by actinospores. However, departure from the host cannot be performed by actinospores, and an unspecific attachment to any living surface seems to be a futile strategy for myxozoan transmission stages. Responses to ubiquitous substances like the use of amino acids by *Ichtyophthirius multifiliis* (Haas *et al*. 1999), would lead to arbitrary attachment responses to any material in an aquatic environment.

The discharge signal seems to be inapparent in *Lymnaea* mucus, which might be useful in avoiding attachment to invertebrates including the oligochaete host. Mollusc epithelial mucins possess great amounts of polysaccharides exhibiting amino sugars, glycoproteins, uronic acid and hexose sulphate (Denny, 1983; Kalbe, Haberl and Haas, 2000), analogous to vertebrate mucins. The efficiacy of these glycoconjugates in stimulating discharge of actinospores is doubtful considering our results. In contrast, a vertebrate-specific signal would enable actinospores to avoid attachment to aquatic invertebrates and explain the response to all our effective test preparations. Nevertheless, this factor requires further investigation with other species (e.g. Hirudineans, other aquatic molluscs).

We could refute the assumption that *M. cerebralis* actinospores are able to select hosts through specific recognition in our experimental set-up. A reason not to use a host-specific discharge mechanism could simply be the speed at which the recognition process must take place between this parasite and its host. The strategy of improving transmission by enormous numbers of transmission stages is not only found in myxozoan parasites and might be the consequence of a lack of species-specific invasion. Non-specific host recognition also creates an open backdoor for the acquisition of new host species and the development of new parasite species after transfer to a new environment. This approach certainly raises the question of how the pronounced host specificity of *M. cerebralis* is accomplished. To understand myxozoan host specificity, it is imperative to clarify how a species or genus-specific myxozoan interacts with non-compatible fish species. In particular the fate of the developmental stages after a possible entry into those non-susceptible fish species needs to be examined.

Even in long-known myxozoan species, our knowledge of development and transmission remains fragmentary (Yokoyama, 2003). The elucidation of these mechanisms is important for our understanding of transmission dynamics in the field. Additionally, premature or unspecifically induced discharge, actuated by biochemicals or decoy organisms, may provide an effective strategy to reduce infectivity of both actinospores and the myxosporean spore and reduce parasite burden in the field. Reliable data on these topics, including the isolation of the triggering substances, can only be achieved through biophysical and behavioural surveys by employing experimental approaches using host material and laboratory cycles. Our investigations provide a method to test the discharge capacity of actinospores and suggest that myxozoan actinospores, despite their mass-production, have to employ a highly effective host recognition mechanism. Extensive effort is currently undertaken in our laboratory to find out more about the molecular

characteristics of the mechano-sensitizing chemostimuli.

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