Parasitol Res (2015) 114:1425–1431
DOI 10.1007/s00436-015-4325-2

ORIGINAL PAPER

Evaluation of nystatin isolated from *Streptomyces griseus* SDX-4 against the ciliate, *Ichthyophthirius multifiliis*

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Received: 13 October 2014 / Accepted: 19 January 2015 / Published online: 3 February 2015

Abstract The present study was conducted to evaluate the in vitro and in vivo antiparasitic efficacy of active compounds from the bacterial extracellular products of *Streptomyces griseus* SDX-4 against *Ichthyophthirius multifiliis*. Bioassay-guided fractionation and isolation of compounds with antiparasitic activity were performed on *n*-butanol extract of *S. griseus* yielding a pure bioactive compound, nystatin (Nys), identified by comparing spectral data (EI-MS, ^{1}H NMR, and ^{13}C NMR) with literature values. Results from in vitro antiparasitic assays revealed that Nys could be 100 % effective against *I. multifiliis* theronts and encysted tomonts at the concentration of 6.0 mg L^{-1}, with the median effective concentration (EC_{50}) values of 3.1 and 2.8 mg L^{-1} for theronts and encysted tomonts (4 h), respectively. Results of in vivo test demonstrated that the number of *I. multifiliis* trophonts on the gold fish treated with Nys was markedly lower than the control group at 10 days after exposed to theronts ($p<0.05$). In the control group, 85.7 % mortality was observed owing to heavy *I. multifiliis* infection at 10 days after the exposure. On the other hand, only 23.8 % mortality owing to parasite infection was recorded in the groups treated with the Nys (4.0 and 6.0 mg L^{-1}). In addition, our results showed that the survival and reproduction of *I. multifiliis* tomont exited from the fish were significantly reduced after treated with the 6.0 mg L^{-1} Nys. The median lethal dose (LD_{50}) of Nys for goldfish was 16.8 mg L^{-1}. This study firstly demonstrated that Nys has potent antiparasitic efficacy against *I. multifiliis*, and it can be a good candidate drug for chemotherapy and control of *I. multifiliis* infections.

Keywords *Ichthyophthirius multifiliis* · Nystatin · *Streptomyces griseus* · Antiparasitic

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Introduction

Ichthyophthirius multifiliis (Ich) is a holotrichous protozoan that invades the gills and skin surfaces of freshwater fish causing the disease ichthyophthiriosis, commonly referred to as ich or white spot. It can cause severe morbidity and high mortality in most species of freshwater fish worldwide and can result in heavy economic losses for aquaculture (Traxler et al. 1998). The life stages of the parasite include an infective theront, a parasitic trophont and a reproductive tomont (Dickerson 2006; Matthews 2005).

Chemical treatment and vaccination have been used to control outbreaks of Ich. The most effective control of Ich infections has been achieved by use of malachite green, a compound that has been banned on fish farms due to its confirmed carcinogenicity in most countries (Alderman 1985; Wahli et al. 1993). Other chemotherapeutants, such as formalin (Rowland et al. 2009), chlorophyllin (Wohllebe et al. 2012), chloramine-T (Rintamäki-Kinnunen et al. 2005), copper sulfate (Rowland et al. 2009), potassium ferrate (VI) (Ling et al. 2011), potassium permanganate (Straus and Griffin 2002), tricaine methanesulfonate (Xu et al. 2008), and bronopol (Shinn et al. 2012) have been evaluated for their antiparasitic efficacy. However, the threats of antiparasitic resistance, risk of residues, environmental contamination, and toxicity to fish caused by the frequent use of these chemicals have led to the

need of other alternative control methods (Goven et al. 1980; Klinger and Floyd 2002). Vaccination against the parasites may provide an alternative to chemical treatment, but it costs highly and this cannot be ignored for fish farmer, restraining its widespread use. There is still an urgent need to develop newly antiparasite agents used in management of Ich disease.

Recently, there have been increased research activities into the utilization of environment-friendly products to control *I. multifiliis* infection (Yao et al. 2010, 2011; Yi et al. 2012). Effective compounds of natural original expected to be more advantageous than synthetic antiparasitic agent, as they have generally a lower environmental impact and are easily biodegradable. Thus, a great number of biological control microorganisms have been screened and researched, including fungi (Crump et al. 1983; Chen and Dickson 1996), bacteria (Becker et al. 1988), ray fungi, and protozoan (Chen and Liu 2005; Sun et al. 2006). *Streptomyces* are distributed widely in terrestrial and marine habitats (Pathom-aree et al. 2006) and are of commercial interest due to their unique capacity to produce novel metabolites. The genus *Streptomyces* was classified under the family Streptomycetaceae, which includes gram-positive aerobic members of the order Actinomycetales and suborder Streptomycineae within the new class Actinobacteria (Anderson and Wellington 2001). They produce approximately 75 % of commercially and medically useful antibiotics and 60 % of antibiotics used in agriculture (Watve et al. 2001). In our previous study, we investigated the anti-*I. multifiliis* efficacy of extracellular products of *Streptomyces griseus* SDX-4 against *I. multifiliis* and found that the *n*-butanol extracts of *S. griseus* (NBu-E) was effective against *I. multifiliis* (Yao et al. 2014). The present study was conducted to assess the antiparasitic properties of NBu-E and isolate active constituents responsible for the activity using in vitro antiparasitic assay associated with bioassay-guided fractionation. Additionally, the acute toxicity of the active compounds against goldfish (*Carassius auratus*) was evaluated.

Materials and methods

Fish and parasites

Parasite-free goldfish were obtained from commercial fish farm and maintained in a 1-m^3 tank supplied with filtered groundwater. The skin surface and gills of ten randomly sampled fish were examined under a microscope to confirm that fish were not infected with gill parasites or skin parasites before the experiments. The fish were fed two times a day at a feeding rate of 1 % body weight (BW). All fish were acclimatized to laboratory conditions for 7 days before the experiment.

The sources of *I. multifiliis*, its propagation on grass carp, and collection of the cysts have been described by Yao et al. (2014). Several heavily infected grass carp (*Ctenopharyngodon idella*) obtained from aquatic fry farm of Zhejiang Institute Freshwater Fisheries in China were placed into filtered aquarium water for 30–60 min. Mature trophonts were allowed to dislodge from the host by body movements of the fish while in close proximity. Isolated trophonts were randomly divided into two batches, one was used to assay the activity of the fraction and active compound isolated from NBu-E for killing the tomonts, and the other placed in plastic beaker with aerated groundwater filtered and incubated for 24 h at 23 °C. The water containing the hatched theronts in the plastic beaker was agitated, and 50 μL of the water was withdrawn five times. The number of theronts in each 50 μL of water was counted, and the average number of parasites per milliliter was calculated to estimate the total number of hatched theronts.

In vitro antiparasitic efficacy against the theronts and tomonts

Tests against *I. multifiliis* theronts were performed as described in previous work (Yao et al. 2010; Ling et al. 2012). Briefly, approximately 100 viable theronts were distributed to each well of 24-well tissue culture plates (Becton Dickinson Labware, NJ, USA) and exposed to different concentrations fractions and active compound from NBu-E. Mortality of theronts of each well was recorded by microscopic examination (×100 magnifications) at 10 min, and 1, 2, 3, and 4 h after exposure. A negative control was included using aerated groundwater containing the same amount of DMSO as the maximum concentration test group. The trial was repeated three times.

For the tomont trials, 30 tomonts were placed into each well of a 24-well tissue culture plate. One-milliliter solutions with different concentrations of test sample were added to each well, respectively. A negative control was included using aerated seawater containing the same amount of DMSO as the maximum concentration test groups. The solutions were replaced by aerated groundwater with no test sample after 4-h exposure. Then, the plates were incubated at 23 °C throughout the trial. The trial was allowed to stop until the parasites in the controls reached the theront stage. At the end of the trial, the dead tomonts was recorded and the mortality was counted. The parasites with the absence of internal cell motility or abnormal cell division and the ones cannot produce the theronts were considered dead. All treatments and control groups were conducted with three replicates.

Bioactivity-guided isolation of pure compound from NBu-E

Strain SDX-4 was inoculated in meat extract (D-glucose 1.0 %, peptone 0.2 %, yeast powder 0.1 %, beef extract 0.1 %, artificial seawater 50 %, pH 7.0–7.2) for 7 days on a reciprocal shaker water bath at 30 °C. The preparation of the *n*-butanol extract of *S. griseus* (NBu-E) was followed by our previous study (Yao et al. 2014). The dried NBu-E were subjected to column chromatography on a silica gel and sequentially eluted with ethyl acetate and methanol with increasing polarity, eventually affording 425 fractions (400 mL each). Thin-layer chromatography (TLC) analysis was performed on silica gel using the same solvent system as the mobile phase. Spots on TLC were visualized under ultraviolet (UV) light (254 and 365 nm) or by spraying the plates with ethanol-sulfuric acid reagent, and fractions showing similar chromatograms were combined into five fractions (Fr. A, 1~65 fractions; Fr. B, 66~128 fractions; Fr. C, 129~230 fractions; Fr. D, 231~315 fractions; Fr. E, 316~425 fractions). These five fractions were submitted to in vitro test, and Fr. D was the most active (median effective concentration (EC_{50}) for theronts and tomonts were 25.6 and 32.8 mg L^{-1}). Fr. D was then applied to macroporous resin HP20 (Lvbaicao Chemical Co., Ltd., Beijing, China) as determined by preliminary experiments and eluted with 10 % methanol (305-nm wavelength, 5.0 mL min^{-1} flow rate, 30 °C column temperature). A crystal was obtained from Fr. D which was isolated by the above method. The structure of the compound was elucidated by comparing spectroscopic data with those reported as nystatin (Thomas 1982; Ling et al. 1986).

In vivo efficacy of Nys against Ich

In vivo test was conducted according to our previous study (Yao et al. 2014) with slight modifications. Briefly, 120 uninfected goldfish, weighing approximately 30 g each, were transferred to 1000-L tanks and were acclimated to laboratory conditions for 7 days before the experiment. After acclimation, approximately 600,000 *I. multifiliis* theronts were put into the 1000-L tanks; the fish were held in the tank for 24 h with gentle aeration to promote infection. After exposure, the fish were divided into four groups: Nys-challenged (2.0, 4.0, 6.0 mg L^{-1}) and control group (challenged with no chemical). Each group consisted of three replicates of 100 L groundwater and ten infected goldfish. The Nys in each tank was replaced on day 3 and day 5 with a fresh solution at the same concentration.

On day 5 after exposure, three fish of each group were placed into 10-L tanks containing filtered aquarium water with no chemical for 45 min; mature parasites were quite freely dislodged from the host by body movements of the fish. Ten glass slides were placed in each tank when fish were transferred to the tanks. Glass slides were collected from the tank of each group, and the number of trophonts was counted under the light microscope. For each treatment, ten trophonts were distributed to each well (N=3) of 24-well tissue culture plate and allowed to attach. The water in each well was removed carefully, and 2 mL of filtered aquarium water was added to each well. Until the parasites in the control groups reached the theront stage, the mortality and reproduction of tomonts in all wells were determined and calculated as described above.

Ten days after exposure to theronts, all remaining fish from each group were randomly sampled and the number of trophonts on the gills and fins was examined. The fish were carefully observed every hour for any signs of distress indicative of erratic behavior, and the fish was pick up to count the trophonts on the gills and fins as soon as dead. Fish mortality was recorded daily, and the parasites on the gills and fins of dead fish were counted.

Acute toxicity of Nys on goldfish

The acute toxicity of Nys was carried out according to the method of Yao et al. (2010) with slight modifications. The tests were conducted in 80-L glass tanks, each containing 50 L of test solution, and ten healthy goldfish. Dilutions were prepared from the stock solution as the following concentrations: 12.0, 14.0, 16.0, 18.0, 20.0, and 22.0 mg L^{-1} for Nys. The tests were conducted in triplicate, as well as controls (under the same test conditions with no chemicals). Fish mortalities in the treatment and control groups were recorded after 48 h of exposure, and median lethal dose (LD_{50}) was calculated.

Data analysis

All data in this study were performed using the SPSS 16.0 probit procedure, and the homogeneity of the replicates of the samples was checked by the Mann–Whitney U test. Tomont survival and tomont reproduction were compared

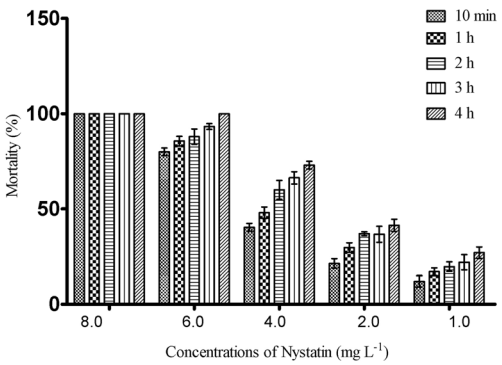

Fig. 1 Antiparasitic efficacy of Nys against *I. multifiliis* theronts after 4-h exposure

Table 1 *I. multifiliis* tomont survival and reproduction after 4-h exposure to Nystatin (mg L^{-1})

Concentrations (mg L^{-1})	Tomont mortality (%)	Reproduction
0 (control)	1.3±1.3 a	488.3±25.7 a
1.0	12.4±2.7 b	463.7±34.3 a
2.0	40.5±4.0 c	325.0±26.5 b
4.0	73.7±4.1 d	107.0±18.1 c
6.0	100±0.0 e	0.0±0.0 d
8.0	100±0.0 e	0.0±0.0 d

The reproduction was represented as number of theronts released by each live tomont. Each value is expressed as mean±standard deviation of three replicates, and within a column, values followed by the different letters are significantly different ($P<0.05$, only compared with control)

with Student–Newman–Keuls test procedure for multiple comparisons ($\alpha=0.05$).

Results

In vitro antiparasitic efficacy of Nys against *I. multifiliis* theronts and tomonts

Nys was separated from Fr. D by bioactivity-guided isolation and was then subjected to in vitro test to determine the active concentrations. The results of Nys against *I. multifiliis* theronts are depicted in Fig. 1. As shown in Fig. 1, Nys concentrations versus *I. multifiliis* theront mortality demonstrated a distinct dose–response and a time–response relationship. The 10-min and 1-, 2-, 3-, and 4-h EC_{50} (95 % confidence interval (CI)) of the compound against theronts of *I. multifiliis* were 4.8 (4.4–5.1), 3.9 (3.6–4.1), 3.5 (3.3–3.7), 3.3 (3.1–3.5), and 3.1 mg L^{-1} (2.8–3.3), respectively.

The results of in vitro trial on the effect of Nys against tomonts are listed in Table 1. All tomonts were dead when exposed to Nys at 6.0 and 8.0 mg L^{-1} concentrations, and no theronts were released. The mortality of tomonts exposed at 4.0, 2.0, and 1.0 mg L^{-1} were 73.7, 40.5, and 12.4 %, respectively. Tested compound led to an obvious dose-dependent lethal effect against tomonts. The 4-h EC_{50} (95 % CI) against tomonts was 2.8 mg L^{-1} (2.5.0–3.1).

In vivo efficacy of Nys against Ich

In the control group, *I. multifiliis* on the body and fins of surviving fish were visible at 3 days and markedly increased at 6 days after starting the trial and increased over the duration of the trial. Mortality was recorded after the exposure due to *I. multifiliis* infection. After starting the trial, 85.7 % fish died at 10 days in the control group (Table 2). In contrast, in the groups treated with Nys, *I. multifiliis* were observed on the body and fins of surviving fish at 4 days after starting the trial, and the number of parasites was significantly lower than that of the control group (Table 2). Furthermore, the number of visible *I. multifiliis* on the body surface of *I. multifiliis* in the group treated with the Nys decreased over time. Among the remaining fish, 23.8 % mortality was recorded at 10 days after the exposure (6.0 mg L^{-1} Nys) due to *I. multifiliis* infection; the average number of *I. multifiliis* trophonts on the gills and fins was 45.7±4.3 (mean±standard deviation). Furthermore, Nys at concentration of 6.0 mg L^{-1} significantly decreased *I. multifiliis* tomont survival and reproduction when the tomonts were collected from infested fish bathed for 5 days, whereas there was no significant difference in tomont survival and reproduction between the fish in the control groups and the fish treated with 2.0 and 4.0 mg L^{-1} Nys.

Acute toxicity of Nys

The fish tolerated Nys at concentration of 12.0 mg L^{-1} for 48 h without visible effects, but exposure to 22.0 mg L^{-1} resulted in an increased opercular movement and erratic behavior of fish within 4 h; all fish died in tanks treated with 22.0 mg L^{-1} of Nys. The linear equation $y=-10.5+12.6x$ was derived from the regression analysis of probit mortality of goldfish in test solution bioassay, and the calculated LD_{50} of Nys was 16.8 mg L^{-1} with the 95 % CI of 16.1–17.3 mg L^{-1}. There was no fish mortality occurred in the control groups during the experiments.

Table 2 In vivo efficacy of Nystatin against Ich

Concentration (mg L^{-1})		Tomonts obtained at day 5		Fish mortality (%)	Number of trophonts in each fish (gills and fins)
		Tomont mortality (%)	Reproduction		
0 (control)		0.0±0.0 a	254.5±23.1 a	85.7	332.5±43.3 a (no. of fish=13)
Nystatin	2.0	75.2±6.3 b	247.7±32.0 a	57.1	206.0±38.5 b (no. of fish=15)
	4.0	56.8±4.2 c	228.3±36.2 a	23.8	58.3±21.2 c (no. of fish=14)
	6.0	45.7±4.3 c	172.6±22.3 b	23.8	43.0±12.5 c (no. of fish=15)

The reproduction was represented as number of theronts released by each live tomont. Each value is expressed as mean±standard deviation of three replicates, and within a column, values followed by the different letters are significantly different ($P<0.05$)

Discussions

The disease ichthyophthiriasis or "white spot," caused by a parasitic ciliate, *I. multifiliis*, probably accounts for more damage to freshwater fish populations worldwide than any other eukaryote pathogen (Matthews 2005). Currently there is no chemotherapeutant available to treat Ich effectively and economically. There is an urgent need to discover effective and safe parasiticides to control ichthyophthiriasis. In the present study, an anti-*I. multifiliis* compound was separated from the *n*-butanol extracts of *S. griseus* by bioactivity-guided isolation and identified as Nys. In vitro tests exhibited that Nys were 100 % effective against *I. multifiliis* theronts and encysted tomonts at concentrations of 6.0 mg L^{-1}. The in vivo test showed that bath treatments with Nys resulted in a significant reduction in the *I. multifiliis* burden of gold fish. Consequently, the parasite-induced mortality of the fish host was significantly reduced. To the best of our knowledge, it seems to be the first report demonstrating the protection efficacy of Nys from parasite infection for fish. This result extended the general knowledge about the antiparasitic activity of Nys to control fish parasite. It also highlights a new way to explore novel active compounds from bacterial extracellular products to combat fish disease.

Nys, produced by *Streptomyces noursei*, was the first polyene macrolide antifungal antibiotic to be discovered (Hazen and Brown 1950). Nys possesses a broad spectrum with both antifungal and fungistatic activity (Recamier et al. 2010) being effective against azole-resistant strains of *Candida* and amphotericin B-resistant strains of *Candida albicans* (Ellepola and Samaranayake 1999). Nys is indicated for treatment of cutaneous and mucocutaneous fungal infections caused by *Candida* species, the main yeast capable of infecting the oral mucosa, being *C. albicans* the most common species isolated (Campos et al. 2012). The antifungal activity of polyene antibiotics is related to their ability to form micelles with ergosterol molecules present in the cell membranes of fungal cells. This bonding causes structural damage and membrane permeabilization leading to the loss of electrolytes and other cytoplasmatic components like proteins. As a consequence of which the fungal cells die (Coutinho and Prieto 2003). Thus, it is postulated that the antiparasitic mechanisms of action of Nys might also be attributed to these factors; however, the exact mechanism of action regarding its antiparasitic efficacy remains to be further investigated.

Theront stage is external elements in the life cycle of *I. multifiliis* (Buchmann et al. 2001). Killing the parasite at this stage will prevent its invasion of fish. It is therefore relevant to assess the susceptibility of free-living theronts to alternative substances. Most theronts (95.3 %) can survive in water for 48 h (Shinn et al. 2012) and possess an increased propensity to infect fish, especially when fish are raised at a high density. Rapidly eliminating theronts can prevent Ich infestation of host fish. This study showed that Nys could 100 % kill the theront at the concentration of 6.0 mg L^{-1}. The exposure duration of Nys was much shorter than garlic extract which required 15 h to eradicate all theronts at a concentration of 62.5 mg L^{-1} (Buchmann et al. 2003) and shorter than potassium ferrate, which took 30 min to eliminate all theronts at a concentration of 24 mg L^{-1} (Ling et al. 2010). The EC_{50} of two active compounds were also lower than some plant compounds, such as dihydrosanguinarine (13.299 mg L^{-1}) and dihydrochelerythrine (18.231 mg L^{-1}) (Yao et al. 2011). Thus, Nys could effectively eradicate theronts in water.

Tomonts are another free-living stage of the *I. multifiliis* life cycle, and each tomont reproduces hundreds to thousands of infective theronts (Matthews 2005; Dickerson 2006). *I. multifiliis* infection can be easily amplified under practical fish farming conditions. So, it is important to prevent infestation of Ich via termination of the reproduction of tomonts. Therefore, a 4-h exposure time for mortality of tomonts was employed in the present study. The results revealed that Nys could be 100 % effective against *I. multifiliis* encysted tomonts at the concentration of 6.0 mg L^{-1}, with the EC_{50} values of 3.1 and 2.8 mg L^{-1} for theronts and encysted tomonts (4 h). Buchmann et al. (2003) revealed that for *I. multifiliis*, tomonts were more resistant to the parasiticides than theronts. This conclusion was supported by other studies (Yi et al. 2012; Ling et al. 2012). But interestingly, in contrast to their findings, we found that EC_{50} value of the Nys against tomonts was closed to theronts. This may because the action site of Nys on tomonts and theronts is coordinate. However, the exact mechanism remains to be further investigated.

Under practical fish farming conditions, it would be necessary to apply drugs repeatedly because tomocysts release new infective theronts continuously over the course of several days. Li and Buchmann (2001) reported that the mean time between tomocyst formation and the release of theronts was found to be 18 h at 25 °C, so Nys was administrated every 2 days in the present study. In vivo test showed that fish treated with Nys at the concentrations of 6.0 mg L^{-1} carried significantly fewer parasites than the control. The observed reduction of parasites in the test groups could be attributed to the effects of the plant extracts because similar reduction in parasite burden was not observed in the control groups. These results suggest that continuous immersion with Nys at the concentration of 6.0 mg L^{-1} every 2 days for three times is suitable for controlling *I. multifiliis* infection.

In the present study, *n*-butanol extract of extracellular products of *S. griseus* was provided for bioactivity-guided isolation. The Fr. D from NBu-E with the most promising activity was subjected to further separation and purification, and the active compound was isolated and identified as Nys. The other extract or fractions with lower antiparasitic activity were not further isolated, although they may contain compounds that

have high activity but present in low concentration. So, further phytochemical studies toward the isolation and separation of the plant are recommended in our future research.

In conclusion, using the strategy of bioactivity-guided isolation monitoring the chromatographic separation, a compound showing promising anti-*I. multifiliis* activity were obtained from NBu-E and elucidated as Nys. It can be chosen as a lead compound for the development of new antiparasitic agents against *I. multifiliis*. Also, further studies are required for field evaluations in the practical system and the mechanism of the antiparasitic activity remains to be performed.

Acknowledgments The research was supported by the National Natural Science Foundation of China (31302211), the Commonness and Commonweal Technology Application project program of Zhejiang province (2014C32055), and the key project program of Huzhou City (2012GN12).

References

Alderman DJ (1985) Malachite green: a review. J Fish Dis 8:289–298

Anderson AS, Wellington EM (2001) The taxonomy of Streptomyces and related genera. Int J Syst Evol Microbiol 51:797–814

Becker JO, Zavaleta ME, Colbert SF, Schroth MN, Weinhold AR, Hancock JG, Vangundy SD (1988) Effects of rhizobacteria on root knot nematodes and gall formation. Phytopathology 78:1466–1469

Buchmann K, Sigh J, Nielsen CV, Dalgaard M (2001) Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis*. Vet Parasitol 100:105–116

Buchmann K, Jensen PB, Kruse KD (2003) Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* theronts and tomocysts: in vitro experiments. N Am J Aquacult 65:21–24

Campos FF, Calpena Campmany AC, Delgado GR, Serrano OL, Naveros BC (2012) Development and characterization of a novel nystatin-loaded nanoemulsion for the buccal treatment of candidosis: ultrastructural effects and release studies. J Pharm Sci 101:739–3752

Chen SY, Dickson DW (1996) Fungal penetration of the cyst wall of Heterodera glycines. Phytopathology 86:319–327

Chen SY, Liu XZ (2005) Control of the soybean cyst nematode by the fungi *Hirsutella rhossiliensis* and *Hirsutella minnesotensis* in greenhouse studies. Biol Control 32:208–219

Coutinho A, Prieto M (2003) Cooperative partition model of nystatin interaction with phospholipid vesicles. Biophys J 84:3061–3078

Crump DH, Sayre RM, Young LD (1983) Occurrence of nematophagous fungi in cysts. Plant Dis 67:63–64

Dickerson HW (2006) *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (Phylum). In: Woo PTK (ed) Fish diseases and disorders. Protozoan and metazoan infections, vol 1, 2nd edn. CAB International, Wallingford, pp 116–153

Ellepola AN, Samaranayake LP (1999) The in vitro post-antifungal effect of nystatin on Candida species of oral origin. J Oral Pathol Med 28: 112–116

Goven B, Gilbert J, Gratzek J (1980) Apparent drug resistance to the organophosphate dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate by monogenetic trematodes. J Wildl Dis 16(3):343–346

Hazen EL, Brown R (1950) Two antifungal agents produced by a soil actinomycete. Science 112:423

Klinger R, Floyd RF (2002) Introduction to freshwater fish parasites. Document CIR716. Institute of Food and Agricultural Science, University of Florida, Florida

Li AH, Buchmann K (2001) Temperature and salinity dependent development of a Nordic strain of *Ichthyophthirius multifiliis* from rainbow trout. J Appl Ichthyol 17:273–276

Ling DK, Chen S, Wang SW, SUN ZP, Ma JW (1986) Identification of two major components in Chinese nystatin. Acta Pharm Sin 21(6): 454–457

Ling F, Wang JG, Liu QF, Li M, Ye LT, Gong XN (2010) Prevention of Ichthyophthirius multifiliis infestation in goldfish (*Carassius auratus*) by potassium ferrate (VI) treatment. Vet Parasitol 168: 212–216

Ling F, Wang JG, Lu C, Wang GX, Lui YH, Gong XN (2012) Effects of aqueous extract of *Capsicum frutescens* (Solanaceae) against the fish ectoparasite *Ichthyophthirius multifiliis*. Parasitol Res 111: 841–848

Ling F, Wang JG, Wang GX, Gong XN (2011) Effect of potassium ferrate(VI) on survival and reproduction of *Ichthyophthirius multifiliis* tomonts. Parasitol Res 109:1423–1428

Matthews RA (2005) *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. Adv Parasitol 59:159–241

Pathom-aree W, Stach JEM, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006) Diversity of actinomycetes isolated from challenger deep sediment (10,898 m) from the Mariana Trench. Extremophiles 10:181–189

Recamier KS, Hernandez-Gomez A, Gonzalez-Damian J, Ortega-Blake I (2010) Effect of membrane structure on the action of polyenes: I. Nystatin action in cholesterol and ergosterol-containing membranes. J Membr Biol 237:31–40

Rintamäki-Kinnunen P, Rahkonen M, Mannermaa-Keränen AL, Suomalainen LR, Mykrä H, Valtonen ET (2005) Treatment of ichthyophthiriasis after malachite green. I. Concrete tanks at salmonid farms. Dis Aquat Organ 64:69–76

Rowland SJ, Mifsud C, Nixon M, Read P, Landos M (2009) Use of formalin and copper to control ichthyophthiriosis in the Australian freshwater fish silver perch (*Bidyanus bidyanus Mitchell*). Aquacult Res 40:44–54

Shinn P, Camacho AP, Bron SM, Conway JE, Yoon D, Guo GH, Taylor FC, Ni GH (2012) The anti-protozoal activity of bronopol onthe key life-stages of *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliophora). Vet Parasitol 186:229–236

Straus DL, Griffin BR (2002) Efficacy of potassium permanganate in treating ichthyophthiriasis in channel catfish. J Aquat Anim Health 14(2):145–148

Sun MH, Gao L, Shi YX, Li BJ, Liu XZ (2006) Fungi and actinomycetes associated with Meloidogyne spp. eggs and in China and their biocontrol potential. J Invertebr Pathol 93:22–28

Thomas AH (1982) The heterogeneous composition of pharmaceutical grade nystatin. Analyst 20:294

Traxler GS, Richard J, McDonald TE (1998) *Ichthyophthirius multifiliis* (Ich) epizootics in spawning sockeye salmon in British Columbia, Canada. J Aquat Anim Health 10:143–151

Wahli T, Schmitt M, Meier W (1993) Evaluation of alternatives to malachite green oxalate as a therapeutant for *ichthyophthiriosis* in rainbow-trout, *Oncorhynchus mykiss*. J Appl Ichthyol 9:237–249

Watve MG, Tickoo R, Jog MM, Bhole BD (2001) How many antibiotics are produced by the genus Streptomyces. Arch Microbiol 176:386–390

Wohllebe S, Richter P, Häder DP (2012) Chlorophyllin for the control of *Ichthyophthirius multifiliis* (Fouquet). Parasitol Res 111:729–733

Xu DH, Shoemaker CA, Klesius PH (2008) Effect of tricaine methanesulfonate on survival and reproduction of the fish ectoparasite *Ichthyophthirius multifiliis*. Parasitol Res 103:979–982

Yao JY, Shen JY, Li XL, Xu Y, Hao GJ, Pan XY, Wang GX, Yin WL (2010) Effect of sanguinarine from the leaves of *Macleaya cordata*

against *Ichthyophthirius multifiliis* in grass carp (*Ctenopharyngodon idella*). Parasitol Res 107:1035–1042

Yao JY, Zhou ZM, Li XL, Yin WL, Ru HS, Pan XY, Hao GJ, Xu Y, Shen JY (2011) Antiparasitic efficacy of dihydrosanguinarine and dihydrochelerythrine from *Macleaya microcarpa* against *Ichthyophthirius multifiliis* in richadsin (*Squaliobarbus curriculus*). Vet Parasitol 183:8–13

Yao JY, Li XC, Li G, Xu Y, Ai WM, Shen JY (2014) Anti-parasitic activities of specific bacterial extracellular products of *Streptomyces griseus* SDX-4 against *Ichthyophthirius multifiliis*. Parasitol Res 113(8):3111–3117

Yi YL, Lu C, Hu XG, Ling F, Wang GX (2012) Antiprotozoal activity of medicinal plants against *Ichthyophthirius multifiliis* in goldfish (*Carassius auratus*). Parasitol Res 111:1771–1778