Original Article

DOI: 10.5582/ddt.2025.01026

Silkworm (*Bombyx mori*) as a novel infection model for fish-derived *Aeromonas hydrophila*

Atsushi Miyashita[1], Kazuhiro Mikami[1,2], Hiroto Nakajima[1], Yidong Yu[1], Masanobu Miyauchi[1,3], Kazuhisa Sekimizu[4,*]

[1] Institute of Medical Mycology, Teikyo University, Tokyo, Japan;
[2] Graduate School of Medical Care and Technology, Teikyo University, Tokyo, Japan;
[3] Department of Psychology, Faculty of Liberal Arts, Teikyo University, Tokyo, Japan;
[4] School of Pharma-Science, Teikyo University, Tokyo, Japan.

SUMMARY: *Aeromonas hydrophila* is a significant pathogenic bacterium in aquaculture and the ornamental fish industry, causing lethal infections in fish and contributing to rising drug resistance. This leads to substantial economic losses and underscores the urgent need for new treatments and infection controls. However, the lack of a simple, sensitive infection model has hindered studies on the pathogenicity of *A. hydrophila* and therapeutic evaluation. This study introduces the silkworm (*Bombyx mori*) as a highly sensitive and cost-effective infection model for *A. hydrophila*. Using a strain isolated from diseased Wakins (goldfish), the pathogenicity of *A. hydrophila* was confirmed in silkworms, which exhibited a much lower median lethal dose (LD_{50} = 0.3 CFU/larva) compared to Wakins (LD_{50} = 5.1 × 10^6 CFU/g body weight). This demonstrates the silkworm's higher sensitivity to *A. hydrophila*. The *in vivo* efficacy of three antibiotics (gentamicin, kanamycin, and tetracycline) was also tested. Gentamicin and kanamycin prolonged survival in both models, while tetracycline also showed efficacy in both models, though its effect was weaker in the silkworm model. This highlights the silkworm model's utility in evaluating bactericidal agents against *A. hydrophila*. This model addresses key limitations of traditional fish infection models, including low sensitivity, long experimental durations, and high costs. The silkworm-based method enables efficient investigation of *A. hydrophila* pathogenicity and rapid screening of potential treatments, accelerating the development of new therapeutic strategies for aquaculture and beyond.

Keywords: Alternative models, silkworm, *Aeromonas*, fish infection, antibiotics

1. Introduction

Infectious disease research relies heavily on animal models to evaluate pathogenicity and develop therapeutic strategies. Traditionally, mammalian models such as mice and rats have been extensively used. However, they entail high costs, require specialized housing facilities, and raise ethical concerns related to animal welfare (*1,2*). Similarly, fish models like zebrafish are valuable for studies in aquatic toxicology and infectious diseases but necessitate specialized equipment for husbandry, limiting the scalability of experiments (*3,4*). These limitations underscore the urgent need for alternative infection models that are cost-effective, ethically acceptable, and easy to manipulate (*1,5*).

The silkworm (*Bombyx mori*) has emerged as a promising model that meets these criteria (*6-10*). Its large body size (4-5 cm in length) and ease of handling allow for precise experimental manipulations such as pathogen inoculation, drug administration, and tissue sampling (*11*). Moreover, silkworms can be reared inexpensively and in large numbers within a short timeframe, enabling large-scale experiments and the efficient acquisition of statistically significant data. Infection models using silkworms have been successfully established for human pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, facilitating the identification of virulence factors and the evaluation of antimicrobial drug efficacy (*12-14*). Additionally, the innate immune system of the silkworm shares significant similarities with those of mammals and fish, including Toll receptor-mediated responses, production of antimicrobial peptides, and phagocytosis by hemocytes (*15-17*), making it an excellent model for analyzing pathogen-host interactions.

Building on the successful use of silkworms as models for human pathogenic infections, this versatile animal model holds great potential for application to pathogens

affecting the aquaculture and ornamental fish industries. In these sectors, bacteria of the genus *Aeromonas*, particularly *Aeromonas hydrophila*, are responsible for lethal diseases and substantial economic losses (*18-20*). *A. hydrophila* is a Gram-negative bacterium and an opportunistic pathogen in fish (also in humans). It can cause widespread infections, inducing hemorrhagic septicemia and ulcerative diseases in fish, which lead to decreased productivity (*18*). The emergence of drug-resistant *A. hydrophila* strains has become a serious concern in recent years (*21*), highlighting the urgent need for new treatments and effective antimicrobial agents. To date, the pathogenicity of *A. hydrophila* has been evaluated primarily in fish or rodent models, such as channel catfish (*Ictalurus punctatus*) (*22*), rainbow trout (*Oncorhynchus mykiss*) (*23*), zebrafish (*Danio rerio*) (*24*), and mouse (*Mus musculus*) (*25*). However, these models are constrained by the various challenges, including high costs, ethical concerns, and limitations in scalability. While invertebrate models like the mealworm (*Tenebrio molitor*) (*26*) and the American crayfish (*Pacifastacus leniusculus*) (*27,28*) have been explored, assessments of *A. hydrophila* pathogenicity using these organisms have remained largely qualitative, lacking quantitative measures such as median lethal dose (LD_{50}) determinations.

In this study, we aim to establish a silkworm infection model for fish-derived *A. hydrophila*, enabling a quantitative evaluation of its pathogenicity and facilitating the assessment of candidate antimicrobial therapies for fish. To validate this model, we will first confirm whether *A. hydrophila* isolates from diseased Wakin goldfish (*Carassius auratus*) induce lethal infections in the silkworm. We will then quantify the LD_{50} in silkworms as an indicator of pathogenicity, thereby confirming the suitability of the silkworm model for evaluating potential antimicrobial treatments. Additionally, we will administer antibacterial agents to infected silkworms to evaluate therapeutic efficacy and confirm the model's suitability for assessing antimicrobial treatments. This silkworm-based research represents the first quantitative assessment of fish-derived *A. hydrophila* pathogenicity and antimicrobial efficacy using invertebrate alternative models, highlighting the potential of invertebrate model in aquaculture disease research. By establishing the silkworm as an efficient alternative infection model, we aim to enable large-scale experimentation and accelerate the development of new treatments, ultimately supporting more sustainable aquaculture practices.

2. Materials and Methods

2.1. Experimental animals

Wakin goldfish (*C. auratus*, body weight 1.3 g to 2.1 g) were purchased from a local supplier, Yoshida Fish Company, and Yoshida Aquarium Shop (Tokyo, Japan). The goldfish were maintained at 22℃ to 25℃ and were not fed after purchase and used for experiments on the following day. Eggs of the silkworm (*B. mori*) KINSYU × SHOWA strain were purchased from Ehime-Sanshu (Ehime, Japan) and reared as described previously (*8,9,29*).

2.2. Isolation, identification and preparation of *A. hydrophila*

The *A. hydrophila* strain used in the study was isolated from diseased Wakin goldfish (*C. auratus*) in our laboratory using brain heart infusion (BHI) agar plate, which were then subjected to DNA extraction, polymerase chain reaction (PCR) amplification of the 16S rRNA region, and sequencing (by conventional dideoxy method). Species identification was confirmed through BLAST analysis (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*) using the standard nucleotide BLAST. The search databases were the 16S ribosomal RNA sequences for bacteria and archaea database, and the nucleotide collection (nr/nt). We included the nucleotide collection in addition to the 16S ribosomal RNA database, because 16S ribosomal sequences for some bacterial isolates were only registered in the nucleotide collection while not in the 16S ribosomal RNA database.

For experiments in this study, the obtained *A. hydrophila* strain was cultured overnight at 30℃ in BHI medium with shaking at 200 rpm. Afterwards, the bacterial culture was washed with physiological saline (0.9% NaCl) by centrifugation at 8,400 *g* for 5 minutes, and the pellet was resuspended in an equal volume of saline to the original culture medium. The concentrations of the bacterial suspensions utilized in the experiments was determined based on colony counts on agar plates.

2.3. Phylogenetic analysis

The obtained 16S rRNA gene sequence was aligned with reference sequences retrieved from the NCBI GenBank database using Clustal Omega. The phylogenetic analysis included the following reference strains: *A. media* ATCC33907, *A. hydrophila* ATCC49140, *A. hydrophila* subsp. LMG19562T, and *A. caviae* ATCC15468. Branch lengths represent genetic distances.

2.4. Infection experiments

In the infection experiments, prepared bacterial suspensions were used to infect both goldfish and silkworms. For goldfish, 50 μL of the bacterial suspension was injected intraperitoneally using a 1-mL syringe with a 27-gauge needle. Silkworms received the same volume (50 μL), injected into the hemolymph with a 27-gauge needle as described previously (*30,31*). The

control groups were injected with the same volume of physiological saline. The number of surviving individuals was observed at two days post-infection.

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing involved measuring the minimum inhibitory concentrations (MICs) of antibiotics against *A. hydrophila*. The antibiotics used were gentamicin, kanamycin, tetracycline, and norfloxacin, all of which were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). MICs were measured using the broth microdilution method with twofold serial dilutions as described previously (*14*). Cultures were incubated at 30°C, and bacterial growth was visually inspected after 24 hours.

2.6. Therapeutic efficacy of selected antibiotics in goldfish (Wakin) and silkworm models

Among the four antibiotics, norfloxacin was poorly soluble in physiological saline to deliver in the host animals. Hence, we excluded norfloxacin from the *in vivo* therapeutic efficacy evaluation. The infection doses were set at 1×10^7 CFU/g body weight for goldfish and 1×10^2 CFU/larva for silkworms, based on the earlier pathogenicity assessment. Each of the three antibiotics (*i.e.*, gentamicin, kanamycin, tetracycline) was dissolved in physiological saline at the desired concentration. After bacterial inoculation, Wakin goldfish and silkworms (n = 4 for each dose) received 50 µL of the antibiotic solution, ether intraperitoneally (for Wakin) or into the hemolymph (for silkworms). Control groups were administered an equivalent volume of physiological saline. The number of survivors was recorded two days post-infection.

2.7. Data analysis and figure drawing

Data analysis was conducted using R (version 4.2.2). We used *dplyr* for data manipulation, *ggplot2* for data visualization, *drc* for dose-response curve analysis, and *readxl* for data import. Dose-response curves were fitted with the *drm* function in *drc*, using a four-parameter logistic model (LL.4). The upper and lower limits were fixed at 1 and 0, respectively. Median lethal (LD_{50}) and effective (ED_{50}) doses were estimated using the ED function. Plots of dose-response curves and other visualization were generated with ggplot2. All presentation materials including graphs and photos were organized using Affinity Designer.

3. Results

3.1. Silkworms exhibit an extreme susceptibility to *A. hydrophila* infection

The *A. hydrophila* strain used in this study (Figure 1a) was isolated from diseased Wakin goldfish (*C. auratus*), with the species identification using BLAST analysis of 16S ribosomal RNA gene sequence (see Figure 1b for phylogenetic tree). The pathogenicity of the isolated *A. hydrophila* strain was evaluated by infecting Wakin goldfish and silkworms (*B. mori*) with the pathogen. Infection resulted in mortality in both hosts within two days post-inoculation, and the lethality was dose-dependent (Figures 2a and 2b for goldfish, and Figures 2c and 2d for silkworms). The LD_{50} was determined to be 5.1×10^6 CFU (colony-forming units)/g body weight for goldfish and 0.30 CFU/larva (average body weight of the silkworm is approximately 2 g, giving ca. 0.15 CFU/g body weight) for silkworms. These results suggests that the silkworm (*B. mori*) can be used as an alternative model for *Aeromonas* infection, with a million-fold susceptibility that enables a rapid and efficient screening for antimicrobials.

3.2. Antibiotic susceptibility testing of isolated *A. hydrophila*

To perform a validation study to confirm therapeutic

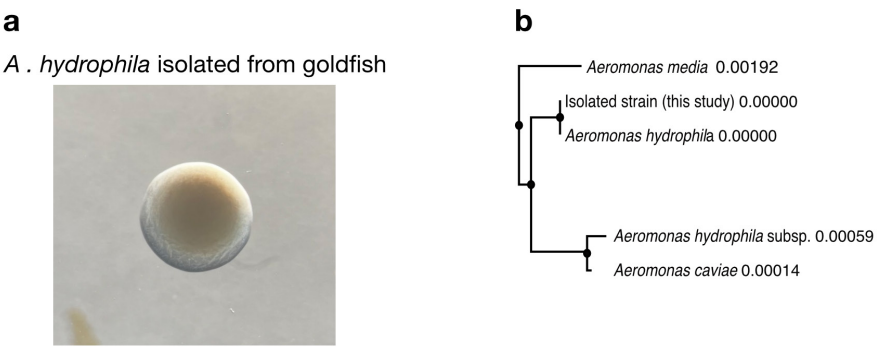

Figure 1. The Aeromonas hydrophila strain isolated in this study. a: Microscopic image of *A. hydrophilia* colony isolated from diseased goldfish. **b**: Phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between the isolated strain and closely related *Aeromonas* species. The isolated strain exhibits 100% identity with the *A. hydrophila*. The phylogenetic analysis includes *A. media* ATCC33907, *A. hydrophila* ATCC49140, *A. hydrophila* subsp. LMG19562T, and *A. caviae* ATCC15468. Branch lengths represent genetic distances.

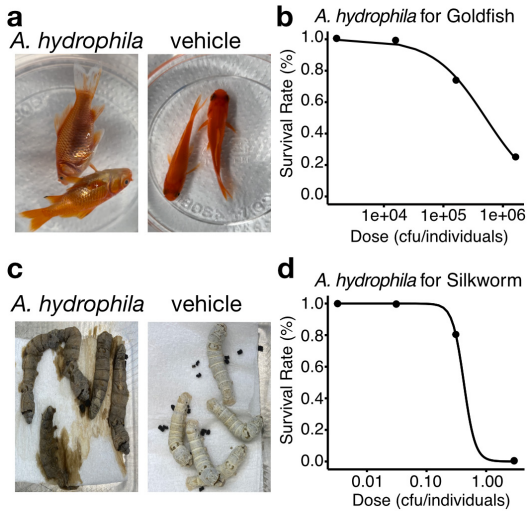

Figure 2. Infection susceptibility of the Wakin goldfish and the silkworm to *A. hydrophila*. **a**: Wakin goldfish succumbed to *A. hydrophila* infection (left panel; infected dose: 6.3×10^6 CFU/g body weight). The right panel shows the control group treated with saline (vehicle). **b**: Dose-response curve showing the survival rate of Wakin goldfish after *A. hydrophila* infection. The x-axis represents the logarithmic bacterial dose, and the y-axis shows the survival rate. Black dots indicate observed survival rates at each dose ($n = 4$). The LD_{50} was calculated as 5.1×10^6 CFU/g body weight. **c**: Silkworms succumbed to *A. hydrophila* infection (left panel; infected dose: 93 CFU/larva). The right panel shows the control group treated with saline (vehicle). **d**: Dose-response curve showing the survival rate of silkworms after *A. hydrophila* infection. The x-axis represents the logarithmic bacterial dose, and the y-axis shows the survival rate. Black dots indicate observed survival rates at each dose ($n = 4$). The LD_{50} was calculated as 0.3 CFU/larva.

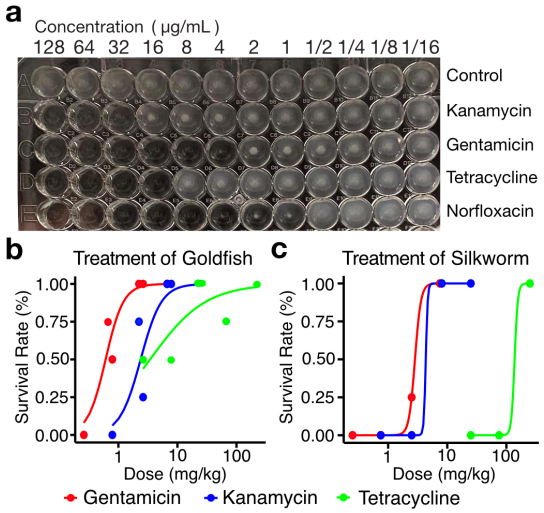

Figure 3. Comparative study on antimicrobial chemotherapy in the goldfish and the silkworm models. **a**: The antimicrobial susceptibility of the isolated *A. hydrophila* strain was evaluated using the broth microdilution method. Bacterial growth was assessed based on turbidity, with clear wells indicating growth inhibition. The MIC values (Table 1) were defined as the lowest antibiotic concentrations that completely inhibited visible bacterial growth. **b**: Therapeutic effects of antimicrobial agents on Wakin goldfish infected with *A. hydrophila*. The y-axis shows survival rates, and the x-axis indicates antibiotic concentration ($n = 4$). ED_{50} values were 0.6 mg/kg for gentamicin, 2.4 mg/kg for kanamycin, and 3.4 mg/kg for tetracycline. **c**. Therapeutic effects of antimicrobial agents on silkworms infected with *A. hydrophila*. The y- axis shows survival rates, and the x-axis indicates antibiotic concentration ($n = 4$). ED_{50} values for these antibiotics were 2.8 mg/kg for gentamicin, 4.3 mg/kg for kanamycin, and 137.6 mg/kg for tetracycline.

efficacy of known antimicrobials using the silkworm-based *A. hydrophila* infection model, we first tested four antimicrobials for their antibacterial activities to the obtained *A. hydrophila* strain. The MICs of the four antibiotics (kanamycin, gentamicin, tetracycline, and norfloxacin) were 32 μg/mL for kanamycin, 4.0 μg/mL for gentamicin, 16 μg/mL for tetracycline, and 1.0 μg/mL for norfloxacin (Figure 3a). These results indicate that the isolated *A. hydrophil*a strain is highly sensitive to gentamicin and norfloxacin but shows lower sensitivity to kanamycin and tetracycline.

3.3. Gentamicin shows the highest therapeutic efficacy against *A. hydrophila* infection in both goldfish and silkworm models

3.3.1. Goldfish

After *A. hydrophila* infection, six different doses of each antibiotic were given to the host animal. All infected goldfish treated with physiological saline died within 24 hours, whereas survival rates in antibiotic-treated groups improved in a dose-dependent manner (Figure 3b). The median ED_{50} for each antimicrobial is listed in Table 1. Gentamicin demonstrated the highest efficacy, with the lowest ED_{50} value of 0.6 mg/kg. In comparison, kanamycin and tetracycline exhibited ED_{50} values of 2.4 mg/kg and 3.4 mg/kg, respectively. These findings highlight gentamicin as the most effective antibiotic against *A. hydrophila* infections in goldfish, requiring the lowest dose to achieve therapeutic effects.

3.3.2. Silkworms

The therapeutic efficacy of the three antibiotics was also evaluated using the silkworm infection model. Following *A. hydrophila* infection, silkworms treated with physiological saline experienced 100% mortality, whereas antibiotic-treated groups (four different doses for each antibiotic, except for tetracycline, which were tested at three doses) showed improved survival in a dose-dependent manner (Figure 3c). The ED_{50} values are presented in Table 1. Gentamicin and kanamycin showed ED_{50} values of 2.8 mg/kg and 4.3 mg/kg, respectively. In contrast, tetracycline exhibited a significantly higher ED_{50} of 138 mg/kg. These results suggest that gentamicin and kanamycin (both bactericidal aminoglycoside antibiotics) are more effective than tetracycline (a bacteriostatic antibiotic) in the silkworm model of *A. hydrophila* infection.

3.3.3. Comparison of the two models

Table 1. Median effective doses (ED_{50}) and ED_{50}/MIC (minimal inhibitory concentration) ratios of antimicrobial agents in the goldfish and silkworm infection models of *A. hydrophila*

Antibiotics	ED_{50} (mg/kg)		MIC (μg/mL)	ED_{50}/MIC ratio	
	Goldfish	Silkworm		Goldfish	Silkworm
Gentamicin	0.6	2.8	4	0.15	0.71
Kanamycin	2.4	4.3	32	0.07	0.14
Tetracycline	3.4	137.6	16	0.21	8.60

The ED_{50}/MIC ratios of gentamicin and kanamycin were relatively small and comparable between the goldfish and silkworm models (0.15 and 0.71 for gentamicin; 0.070 and 0.14 for kanamycin; see Table 1). These findings indicate that gentamicin and kanamycin (bactericidal antibiotics) maintain their efficacy *in vivo*, aligning well with their *in vitro* activity (*12*). In contrast, tetracycline (a bacteriostatic antibiotic) exhibited a markedly higher ED_{50}/MIC ratio in the silkworm model (8.6) compared to the goldfish model (0.21).

4. Discussion

4.1. A silkworm-based novel infection model of *A. hydrophila*

In the present study, we evaluated the pathogenicity and antibiotic susceptibility of *A. hydrophila* using both Wakin goldfish (*C. auratus*) and silkworms (*B. mori*) as infection models. *A. hydrophila* induced lethal infections within two days post-inoculation in both hosts. The median lethal dose (LD_{50}) values were 5.1×10^6 CFU/g body weight for goldfish and only 0.30 CFU/larva for silkworms, underscoring the silkworm model's potential for rapid and efficient *in vivo* screening of anti–*A. hydrophila* compounds.

The use of silkworms offers several advantages over other animal models. Compared with mammalian systems such as mice or rats, silkworms lower both ethical and economic burdens. They also require less specialized maintenance than fish models like zebrafish (*3,4*). Notably, silkworms' pronounced susceptibility to infection enables sensitive and reproducible detection of pathogenicity differences among bacterial strains (*13*), including those with low virulence or lacking key virulence factors. However, because of this extreme sensitivity, the silkworm model may not always distinguish subtle differences in pathogenicity among highly virulent strains. Addressing this issue will require identifying key factors that drive both bacterial pathogenicity and silkworm susceptibility, thereby broadening the utility of this model for analyzing a broader spectrum of pathogenic microbes.

4.2. Correlation of antibacterial effectiveness across models

We next evaluated antibiotic efficacy in both silkworms and goldfish inoculated with *A. hydrophila*. Despite the rapid disease progression in silkworms, dose-dependent therapeutic effects were clearly demonstrated, as survival rates improved with antibiotic administration in both models. These findings confirm that the silkworm model can quantitatively assess antibacterial activity, providing a feasible platform for screening new or existing drugs against fish-associated bacterial pathogens. Because experimental studies can be conducted efficiently and cost-effectively in silkworms, this model has considerable potential for accelerating drug discovery for aquaculture. Moreover, previous reports indicate that the pharmacokinetics (ADME: Absorption, Distribution, Metabolism, Excretion) of many antibiotics in silkworms correlate well with those in mammals (*32*). Therefore, results obtained from the silkworm model may have broader relevance for treating infections in both fish and mammalian systems. Further investigation into pharmacokinetic and pharmacodynamic parameters in silkworms and fish will enhance confidence in the model's translational value for aquaculture and beyond.

Among the three antibiotics tested (gentamicin, kanamycin, and tetracycline), the aminoglycosides (gentamicin and kanamycin) exhibited particularly high efficacy in both goldfish and silkworms. A potential explanation for this finding is linked to the exceptionally high susceptibility of silkworms to *A. hydrophila*, which may reflect a relative lack of immune mechanisms against this pathogen. In other words, silkworms could be considered in an "immunosuppressed" state regarding *A. hydrophila* infection. Because bactericidal antibiotics like aminoglycosides directly kill bacteria, they do not rely as heavily on the host's immune system to clear infection; thus, they provided a marked therapeutic benefit in silkworms. In contrast, bacteriostatic antibiotics such as tetracycline primarily inhibit bacterial growth; full clearance typically requires an intact host immune response to remove nonreplicating microbes. When the host's defense mechanisms are weakened or insufficient (*e.g.*, in silkworms lacking certain vertebrate-like adaptive immune components), bacteriostatic agents may not achieve a sufficient therapeutic effect. This distinction underscores the importance of understanding the host's immune capacity when evaluating antibiotic efficacy, particularly in model organisms with reduced or simplified immune systems.

4.3. Limitation of the study

Despite our findings, the underlying reason for silkworms' extreme susceptibility to *A. hydrophila* remains unclear. One possibility is that, unlike vertebrates, silkworms lack an adaptive immune system, potentially reducing their ability to control bacterial proliferation. Moreover, *A. hydrophila* may produce specific factors that evade or suppress innate immune responses, making silkworms (reliant solely on innate immunity) especially vulnerable to infection. Alternatively, silkworm physiology may offer conditions highly conducive to rapid bacterial growth. Elucidating these mechanisms will provide deeper insights into the virulence strategies of *A. hydrophila* and may inform novel approaches to preventing and treating infections in other hosts.

5. Conclusion

This study demonstrates that both silkworms (*B. mori*) and Wakin goldfish (*C. auratus*) can serve as valuable infection models for evaluating the pathogenicity of *A. hydrophila* and assessing antibiotic efficacy. Silkworms exhibited extreme sensitivity to *A. hydrophila* (an LD_{50} of 0.30 CFU/larva), making them particularly useful for detecting low-pathogenicity strains, compared with goldfish (an LD_{50} of 5.1×10^6 CFU/g body weight). Among the antibiotics tested, aminoglycosides (gentamicin and kanamycin) showed dose-dependent therapeutic effects in both models, highlighting the potential of silkworms for cost-effective, large-scale antibiotic screening. Moreover, the simplicity of silkworm husbandry and reduced ethical concerns further underscore their suitability for infection research. Future studies focusing on pharmacokinetic and pharmacodynamic correlations between silkworms and fish will likely expand the applicability and reliability of the silkworm model, contributing to improved infectious disease control in aquaculture and the development of novel therapeutic strategies.

Acknowledgements

We thank Genome Pharmaceuticals Institute, Inc., for technical assistance for the study. This work was supported by JSPS KAKENHI (Grant# 22K15461, AM), the Research and implementation promotion program through open innovation grants (Grant# JPJ011937, to the consortium where AM serves as a member) from the Project of the Bio-oriented Technology Research Advancement Institution (BRAIN), and a Teikyo University Team Research Grant (Grant# 22–24, AM).

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Kaito C, Murakami K, Imai L, Furuta K. Animal infection models using non-mammals. Microbiol Immunol. 2020; 64:585-592.
2. Walker RL, Eggel M. From mice to monkeys? Beyond orthodox approaches to the ethics of animal model choice. Animals (Basel). 2020; 10:77.
3. Ribas L, Piferrer F. The zebrafish (*Danio rerio*) as a model organism, with emphasis on applications for finfish aquaculture research. Rev Aquac. 2014; 6:209-240.
4. Li H, Liu Y, Chen Q, Jin L, Peng R. Research progress of zebrafish model in aquatic ecotoxicology. Water. 2023; 15:1735.
5. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med. 2005; 22:359-370.
6. Tabuchi F, Mikami K, Miyauchi M, Sekimizu K, Miyashita A. Discovery of new AMR drugs targeting modulators of antimicrobial activity using *in vivo* silkworm screening systems. Antibiot (Tokyo). 2025; 78:69-77.
7. Miyashita A, Kataoka K, Tsuchida T, Ogasawara AA, Nakajima H, Takahashi M, Sekimizu K. High molecular weight glucose homopolymer of broccoli (*Brassica oleracea* var. *italica*) stimulates both invertebrate and mammalian immune systems. Front Food Sci Technol. 2023; 3. DOI: 10.3389/frfst.2023.1012121
8. Miyashita A, Mitsutomi S, Mizushima T, Sekimizu K. Repurposing the PDMA-approved drugs in Japan using an insect model of staphylococcal infection. FEMS Microbes. 2022; 3:xtac014.
9. Li W, Miyashita A, Sekimizu K. Peanut triacylglycerols activate innate immunity both in insects and mammals. Sci Rep. 2022; 12:1-9.
10. Miyashita A, Sekimizu K. Using silkworms to search for lactic acid bacteria that contribute to infection prevention and improvement of hyperglycemia. Drug Discov Ther. 2021; 15:51-54.
11. Kaito C, Akimitsu N, Watanabe H, Sekimizu K. Silkworm larvae as an animal model of bacterial infection pathogenic to humans. Microb Pathog. 2002; 32:183-190.
12. Hamamoto H, Kurokawa K, Kaito C, Kamura K, Manitra Razanajatovo I, Kusuhara H, Santa T, Sekimizu K. Quantitative evaluation of the therapeutic effects of antibiotics using silkworms infected with human pathogenic microorganisms. Antimicrob Agents Chemother. 2004; 48:774-779.
13. Kaito C, Sekimizu K. A silkworm model of pathogenic bacterial infection. Drug Discov Ther. 2007; 1:89-93.
14. Hamamoto H, Urai M, Ishii K, *et al*. Lysocin E is a new antibiotic that targets menaquinone in the bacterial membrane. Nat Chem Biol. 2015; 11:127-133.
15. Taniai K, Wago H, Yamakawa M. *In vitro* phagocytosis of *Escherichia coli* and release of lipopolysaccharide by adhering hemocytes of the silkworm, *Bombyx mori*. Biochem Biophys Res Commun. 1997; 231:623-627.
16. Wu S, Zhang X, Chen X, Cao P, Beerntsen BT, Ling E. BmToll9, an Arthropod conservative Toll, is likely involved in the local gut immune response in the silkworm, *Bombyx mori*. Dev Comp Immunol. 2010;

34:93-96.
17. Cheng T, Zhao P, Liu C, Xu P, Gao Z, Xia Q, Xiang Z. Structures, regulatory regions, and inductive expression patterns of antimicrobial peptide genes in the silkworm *Bombyx mori*. Genomics. 2006; 87:356-365.
18. Chakraborty N, Das BK, Bera AK, Borah S, Mohanty D, Yadav AK, Kumar J, Koushlesh SK, Chanu TN, Panda SP, Vallangi R. Co-prevalence of virulence and pathogenic potential in multiple antibiotic resistant *Aeromonas* spp. from diseased fishes with *in silico* insight on the virulent protein network. Life. 2022; 12:1979.
19. Beaz-Hidalgo R, Figueras MJ. *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. J Fish Dis. 2013; 36:371-388.
20. Hossain S, Dahanayake PS, De Silva BCJ, Wickramanayake M, Wimalasena S, Heo GJ. Multidrug resistant *Aeromonas* spp. isolated from zebrafish (*Danio rerio*): antibiogram, antimicrobial resistance genes and class 1 integron gene cassettes. Lett Appl Microbiol. 2019; 68:370-377.
21. Hossain S, Heo GJ. Ornamental fish: a potential source of pathogenic and multidrug-resistant motile *Aeromonas* spp. Lett Appl Microbiol. 2021; 72:2-12.
22. De Figueiredo J, Plumb JA. Virulence of different isolates of *Aeromonas hydrophila* in channel catfish. Aquaculture. 1977; 11:349-354.
23. Newaj-Fyzul A, Adesiyun AA, Mutani A, Ramsubhag A, Brunt J, Austin B. Bacillus subtilis AB1 controls Aeromonas infection in rainbow trout (Oncorhynchus mykiss, Walbaum). J Appl Microbiol. 2007; 103:1699-706.
24. Romero A, Saraceni PR, Merino S, Figueras A, Tomás JM, Novoa B. The animal model determines the results of *Aeromonas* virulence factors. Front Microbiol. 2016; 7.
25. Wong CYF, Heuzenroeder MW, Flower RLP. Inactivation of two haemolytic toxin genes in *Aeromonas hydrophila* attenuates virulence in a suckling mouse model. Microbiology (Reading). 1998; 144 (Pt 2):291-298.
26. Noonin C, Jiravanichpaisal P, Söderhäll I, Merino S, Tomás JM, Söderhäll K. Melanization and pathogenicity in the insect, *Tenebrio molitor*, and the crustacean, *Pacifastacus leniusculus*, by *Aeromonas hydrophila* AH-3. PLoS One. 2011; 5:e15728.
27. Jiravanichpaisal P, Roos S, Edsman L, Liu H, Söderhäll K. A highly virulent pathogen, *Aeromonas hydrophila*, from the freshwater crayfish *Pacifastacus leniusculus*. J Invertebr Pathol. 2009; 101:56-66.
28. Zeng Y. *Aeromonas hydrophila*, one reason causing the death of freshwater crayfish *Procambarus clarkii* (Girard, 1852). Iran J Fish Sci. 2020; 19:1770-1779.
29. Miyashita A, Hamamoto H, Sekimizu K. Applying the silkworm model for the search of immunosuppressants. Drug Discov Ther. 2021; 15:139-142.
30. Miyashita A, Kizaki H, Kawasaki K, Sekimizu K, Kaito C. Primed immune responses to gram-negative peptidoglycans confer infection resistance in silkworms. J Biol Chem. 2014; 289:14412-14421.
31. Miyashita A, Iyoda S, Ishii K, Hamamoto H, Sekimizu K, Kaito C. Lipopolysaccharide O-antigen of enterohemorrhagic *Escherichia coli* O157:H7 is required for killing both insects and mammals. FEMS Microbiol Lett. 2012; 333:59-68.
32. Panthee S, Paudel A, Hamamoto H, Sekimizu K. Advantages of the silkworm as an animal model for developing novel antimicrobial agents. Front Microbiol. 2017; 8:373.

Received March 27, 2025; Accepted April 15, 2025.

*Address correspondence to:
Kazuhisa Sekimizu, School of Pharma-Science, Teikyo University, Tokyo, Japan.
E-mail: sekimizu@main.teikyo-u.ac.jp

Released online in J-STAGE as advance publication April 27, 2025.