Received: 27 March 2023 | Revised: 4 May 2023 | Accepted: 11 May 2023

DOI: 10.1002/naaq.10300

ARTICLE

Ultraviolet light differentially reduces viability of fish- and fish farm-associated flavobacteria (families Flavobacteriaceae and Weeksellaceae)

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Abstract

Objective: Globally, flavobacteria (family Flavobacteriaceae and Weeksellaceae) are leading causes of disease-related losses in fish-farms and hatcheries. One route flavobacteria gain access to aquaculture facilities is via source water. Ultraviolet (UV) light treatment of source water has been effective in reducing the risk of disease outbreaks caused by nonflavobacteria; however, the UV dose required to inactivate flavobacteria has been understudied. The primary objective of this study was to examine the efficacy of UV light treatments for reducing the viability of fish-pathogenic and fish-associated *Flavobacterium* and *Chryseobacterium* species in a planktonic form.

Methods: Sixty-five flavobacterial isolates belonging to ten *Flavobacterium* spp. and *Chryseobacterium* spp. were exposed to a low (25 mJ/cm^2) and high (126 mJ/cm^2) dose of UV light via a collimating beam apparatus under in vitro conditions, after which treatment efficacy was determined via culture.

Result: All assayed flavobacteria were reduced by an average of ~1000-fold or ~100,000-fold at the low and high UV doses, respectively; however, substantial differences in reduction at the same UV dose were noted among isolates of the same flavobacterial species, including *F. psychrophilum*, *F. columnare*, and *F. oreochromis*.

In addition, *F. psychrophilum* multilocus sequence typing variants ST10 and ST78, which are two of the most widespread variants in the United States of America, were among the least susceptible to ultraviolet light.

Conclusion: Overall, results demonstrate that viable flavobacteria can be reduced substantially by ultraviolet doses of 25–126 mJ/cm^2, suggesting such treatments represent a promising tool for minimizing flavobacterial loads in hatcheries and aquaculture facilities, thereby enhancing biosecurity and reducing the risk of epizootics.

KEYWORDS

bacterial coldwater disease, biosecurity, columnaris disease, flavobacteria, *Flavobacterium*, ultraviolet light

INTRODUCTION

Fish diseases caused by multiple yellow-pigmented bacteria within the family Flavobacteriaceae (Bernardet et al. 1996) are collectively among the top contributors to disease-associated losses in aquaculture and hatchery facilities globally (Loch and Faisal 2017). Among the most common causes of such losses are *Flavobacterium psychrophilum*, the etiological agent of bacterial coldwater disease (BCWD) and Rainbow Trout fry syndrome (Davis 1946; Holt 1987); the agents of columnaris disease (e.g., *F. columnare*, *F. covae, F. davisii*, and *F. oreochromis*; Davis 1922; Bernardet and Grimont 1989; LaFrentz et al. 2022; collectively referred to as "columnaris-causing bacteria"); and *F. branchiophilum*, a cause of bacterial gill disease (Wakabayashi et al. 1989). In addition, multiple seemingly emergent and novel *Flavobacterium* spp. and *Chryseobacterium* spp. (family Weeksellaceae; Garcia-Lopez et al. 2019) have been increasingly linked to disease outbreaks in a range of captive-reared fishes (Loch and Faisal 2015).

In contrast to the diversity of fish-pathogenic flavobacteria is their seemingly unified ability to circumvent current methods of disease prevention and control. For example, iodophor, a widely used fish egg disinfectant, does not completely eradicate flavobacteria on or within infected eggs (Brown et al. 1997; Kumagi et al. 1998; Loch and Faisal 2016, 2018). Additionally, there are reports of reduced susceptibility to the few antibiotics that are approved to treat flavobacterial infections in fish (Bruun et al. 2000; Schmidt et al. 2000; Van Vliet et al. 2017), and the development of efficacious licensed BCWD and columnaris vaccines has proven elusive to date (Bebak and Wagner 2012; Gomez et al. 2014).

Many fish-pathogenic flavobacteria also gain access to fish rearing facilities via source water, particularly those that utilize surface water (Bebak et al. 1997; Wiklund et al. 2000; Madetoja et al. 2002; Kunttu et al. 2012). To minimize the likelihood of introducing fish pathogens via source water (Cross and Peterson 1987; Masters et al. 2018), some aquaculture facilities treat incoming water with ultraviolet (UV) light (Summerfelt 2003). Multiple studies have reported that a UV dose of 30 mJ/cm^2 is effective at inactivating bacterial fish pathogens; therefore, this dose is widely recommended for water disinfection at aquaculture facilities (Wedemeyer 1996; Liltved 2002; Sharrer et al. 2005). However, most of these studies did not use culture media or detection methods that are appropriate for flavobacteria. Among the few studies that have explored the UV doses required to inactivate flavobacteria, results have been inconsistent. Farkas et al. (1986) examined a UV dose of 3×10^{-7} mJ/cm^2 against *F. columnare* occurring naturally in source water and reported that the bacterium remained viable in aquaria receiving the UV-treated water; notably, this treatment was several orders of magnitude lower than UV doses reported in other studies. Elmore (2016) found that a UV dose of 5 mJ/cm^2 achieved a 3.5 log reduction of viable *F. psychrophilum*; conversely, Hedrick et al. (2000) found that a UV dose of 126 mJ/cm^2, which greatly exceeds the 30-mJ/cm^2 dose commonly recommended for aquaculture systems (Wedemeyer 1996; Liltved 2002; Sharrer et al. 2005), was required to achieve a 5 log reduction of a single *F. psychrophilum* isolate. Studies conducted on flavobacteria that were recovered from polar environments have suggested relative resistance to UV exposure (Marizcurrena et al. 2017).

Impact statement

In this study, ultraviolet light effectively reduced multiple fish disease-causing flavobacteria under laboratory conditions. Thus, ultraviolet light treatment of water is a promising tool for reducing harmful flavobacteria in fish farms and hatcheries, thereby potentially improving fish health and aquaculture sustainability.

The disparate UV doses required to inactivate different *F. psychrophilum* isolates could be related to this species' substantial intraspecific diversity. Multilocus sequence typing (MLST) studies have revealed there to be at least 260 *F. psychrophilum* sequence types (STs) worldwide (https://pubmlst.org/organisms/flavobacterium-psychrophilum), some of which appear to differ in host species association (Nicolas et al. 2008; Knupp et al. 2019, 2021a), antimicrobial susceptibility (Van Vliet et al. 2017), serotype (Rochat et al. 2017; Avendaño-Herrera et al. 2020), and virulence (Sundell et al. 2019; Knupp et al. 2021b). Likewise, genetic heterogeneity within *F. columnare*, which was recently emended to be four distinct species (*F. columnare*, *F. covae*, *F. davisii*, and *F. oreochromis*; LaFrentz et al. 2022), has also been associated with phenotypic differences (LaFrentz et al. 2018) and therefore may also contribute to differences in UV light susceptibility.

Fish-pathogenic flavobacteria continue to cause substantial global losses in aquaculture and hatchery-based conservation facilities, and few disparate results on flavobacterial susceptibility to UV light exist. Therefore, this study was designed with the primary objective of determining the UV doses that are capable of efficaciously inactivating a diversity of fish-pathogenic flavobacteria, including columnaris-causing bacteria and an assortment of *F. psychrophilum* MLST variants, under in vitro conditions. In addition, the UV light susceptibility of *Aeromonas salmonicida* subsp. *salmonicida*, *Carnobacterium maltaromaticum*, and *Yersinia ruckeri* was investigated due to their role as fish pathogens and the limited or nonexistent UV light susceptibility data for these taxa (Liltved and LandFald 1996; Wedemeyer 1996).

MATERIALS AND METHODS

Bacterial isolates

Sixty-five flavobacterial isolates were evaluated for UV light susceptibility in this study (Table 1; Table S1 available in the Supplementary Materials in the online version of this article). Thirty-two of the isolates were previously identified as eight *Flavobacterium* spp.: namely, *F. branchiophilum* ($n=1$; Wakabayashi et al. 1989), *F. columnare* ($n=1$; Faisal et al. 2016), *F. covae* ($n=2$; LaFrentz et al. 2022), *F. davisii* ($n=1$; LaFrentz et al. 2022), *F. oreochromis* ($n=2$; LaFrentz et al. 2022), *F. plurextorum* ($n=1$; Zamora et al. 2013), *F. psychrophilum* ($n=23$ isolates in 12 STs; Van Vliet et al. 2016; Knupp et al. 2019), and *F. tructae* ($n=1$; Loch and Faisal 2014a; Kämpfer et al. 2020). Three isolates were previously identified as *Chryseobacterium* spp.: *C. aahli* ($n=1$; Loch and Faisal 2014b), *C. aquaticum* ($n=1$; Kim et al. 2008), and *C. scophthalmum* ($n=1$; VanDamme et al. 1994). The remaining 30 isolates were newly identified as flavobacteria or *F. columnare* using previously published protocols (Loch et al. 2013; LaFrentz et al. 2019).

The 65 flavobacterial isolates were recovered from seven fish genera and 11 species, including Rainbow Trout *Oncorhynchus mykiss* ($n=33$), Chinook Salmon *Oncorhynchus tshawytscha* ($n=4$), Coho Salmon *Oncorhynchus kisutch* ($n=3$), Lake Trout *Salvelinus namaycush* ($n=3$), Brown Trout *Salmo trutta* ($n=2$), Channel Catfish *Ictalurus punctatus* ($n=2$), tilapia *Oreochromis* spp. ($n=2$), Atlantic Salmon *Salmo salar* ($n=1$), Muskellunge *Esox masquinongy* ($n=1$), Largemouth Bass *Micropterus salmoides* ($n=1$), and Turbot *Scophthalmus maximus* ($n=1$; Table S1). Of the remaining 12 isolates, 11 were recovered from hatchery water and one was recovered from a water reservoir (Table S1).

In addition, type strains of three other bacterial fish pathogens—*A. salmonicida* subsp. *salmonicida* (American Type Culture Collection [ATCC] 33658^T), *C. maltaromaticum* (ATCC 35586^T), and *Y. ruckeri* (ATCC 29473^T)—were included in this study (Table 1; Table S1).

Bacterial culture for ultraviolet light susceptibility experiments

Flavobacterium spp. and *Chryseobacterium* spp. were grown using Hsu–Shotts agar/broth (Bullock et al. 1986) or tryptone yeast extract agar/broth (Holt 1987) and were incubated at 15°C or 22°C depending on the isolate. *Aeromonas salmonicida* subsp. *salmonicida*, *C. maltaromaticum*, and *Y. ruckeri* were cultivated using tryptone soya agar/broth (ThermoScientific Oxoid) and were incubated at 22°C.

In preparation for the UV light susceptibility experiment, isolates were revived from cryostock (maintained at −80°C) on the appropriate solid medium, incubated for 72 h at either 15°C or 22°C, and then visually inspected for purity. A 1-μL loopful of each isolate was inoculated into 45 mL of analogous broth and incubated with constant shaking (180 rpm) for 48 h at either 15°C or 22°C. Bacteria were harvested via centrifugation (2571 ***g***, 10 min) and resuspended into sterile saline (i.e., a planktonic bacterial suspension) to an optical density (OD) of 2.0 at 600 nm (OD_{600}) using a Biowave CO8000 Cell Density Meter (i.e., a spectrophotometer; Walden Precision Apparatus). To quantify bacterial concentrations, a 1-mL aliquot was serially diluted up to 100,000,000-fold in 10-fold increments, plated on the appropriate solid medium in duplicate, and then incubated for 7 days at the appropriate temperature, after which final colony counts were performed. In this context, an OD_{600} of 2.0 corresponded to approximately

TABLE 1 Summary information for the 65 flavobacterial isolates and three nonflavobacterial isolates used in this study, including bacterial species, 16S ribosomal RNA (rRNA) percent similarity (newly presented isolates in this study only), multilocus sequence typing sequence type (ST) and clonal complex (CC; *Flavobacterium psychrophilum* only), and $\log_{10}$ reduction of colony-forming units (mean ± SE) at ultraviolet doses of 25 and 126 mJ/cm^2. All bacterial suspensions were adjusted to an optical density of 2.0 at 600 nm. The table is alphabetically arranged by species.

Isolate ID	Species or most similar described	16S rRNA similarity (%)	ST/CC	$\log_{10}$ reduction ± SE	
				25 mJ/cm^2	126 mJ/cm^2
ATCC 33658^T	*Aeromonas salmonicida* subsp. *salmonicida*			3.15 ± 0.15	3.39 ± 0.24
ATCC 35586^T	*Carnobacterium maltaromaticum*			1.50 ± 0.10	3.39 ± 0.09
ATCC BAA-2540^T	*Chryseobacterium aahli*			5.26 ± 0.14	9.54 ± 0.24[a]
KCTC 12483^T	*Chryseobacterium aquaticum*			6.06 ± 0.06	9.30 ± 0.00[a]
NIFA-501	*Chryseobacterium ginsengiterrae*	97.9		4.67 ± 0.15	4.89 ± 0.59
NIFA-230	*Chryseobacterium indoltheticum*	100		3.70 ± 0.00	4.60 ± 0.30
NIFA-441	*Chryseobacterium lactis*	99.2		3.70 ± 0.00	5.24 ± 0.24
NIFA-301	*Chryseobacterium piscium*	99.4		0.98 ± 0.12	2.96 ± 0.04
NIFA-302	*C. piscium*	98.2		2.88 ± 0.24	4.09 ± 0.09
NIFA-589	*C. piscium*	97.6		3.44 ± 0.22	4.85 ± 0.15
NIFA-491-B	*C. piscium*	99.5		3.55 ± 0.15	8.70 ± 0.00[a]
NIFA-214	*C. piscium*	96.4		3.83 ± 0.13	4.29 ± 0.20
NIFA-281	*C. piscium*	98.1		4.06 ± 0.06	5.69 ± 0.09
NIFA-580	*C. piscium*	97.4		4.50 ± 0.20	9.74 ± 0.04[a]
NIFA-224	*C. piscium*	97.6		4.61 ± 0.21	9.65 ± 0.05[a]
NIFA-494	*C. piscium*	92.8		8.81 ± 0.03[a]	9.04 ± 0.00[a]
ATCC 700039^T	*Chryseobacterium scophthalmum*			2.62 ± 0.22	3.82 ± 0.22
NIFA-403	*Flavobacterium aquidurense*	98.6		2.04 ± 0.00	3.15 ± 0.15
NIFA-309	*F. aquidurense*	98.7		3.00 ± 0.00	5.00 ± 0.00[a]
NIFA-303	*F. aquidurense*	96.1		4.39 ± 0.09	5.30 ± 0.00
NIFA-192	*F. aquidurense*	98.3		6.30 ± 0.00[a]	6.82 ± 0.00[a]
NIFA-385	*F. aquidurense*	98.8		9.00 ± 0.00[a]	8.70 ± 0.00[a]
NIFA-478	*Flavobacterium bizetiae*	98.7		2.70 ± 0.00	4.15 ± 0.15
NIFA-475	*Flavobacterium branchiarum*	99.0		4.09 ± 0.09	5.15 ± 0.15
ATCC 35036^T	*Flavobacterium branchiophilum*			2.76 ± 0.24	3.61 ± 0.09
090702-1 3	*Flavobacterium columnare*[b]			3.61 ± 0.21	4.29 ± 0.08
181002-1 10	*F. columnare*[b]			5.54 ± 0.11	9.22 ± 0.04[a]
ALG-00-530	*Flavobacterium covae*[b]			6.54 ± 0.24[a]	8.00 ± 1.00[a]
AL-02-36^T	*F. covae*[b]			7.39 ± 0.09[a]	7.30 ± 0.30[a]
NIFA-204	*Flavobacterium cupreum*	98.4		5.00 ± 0.00	9.15 ± 0.15[a]
90-106^T	*Flavobacterium davisii*[b]			1.37 ± 0.15	3.85 ± 0.15
NIFA-312	*Flavobacterium oncorhynchi*	99.3		1.76 ± 0.24	6.00 ± 0.00[a]
Costa Rica 04-02-TN^T	*Flavobacterium oreochromis*[b]			5.03 ± 0.08	8.78 ± 0.18[a]
BZ-1-02	*F. oreochromis*[b]			1.51 ± 0.16	1.94 ± 0.02
NIFA-255	*Flavobacterium pectinovorum*	98.7		4.00 ± 0.00	5.00 ± 0.00
NIFA-469	*Flavobacterium piscis*	96.4		4.15 ± 0.15	4.46 ± 0.24
NIFA-579	*Flavobacterium plurextorum*	97.0		3.70 ± 0.00	5.39 ± 0.09
CECT 7844^T	*F. plurextorum*			5.63 ± 0.15	7.70 ± 0.00[a]

TABLE 1 (Continued)

Isolate ID	Species or most similar described	16S rRNA similarity (%)	ST/CC	Log_{10} reduction ± SE	
				25 mJ/cm^2	126 mJ/cm^2
ATCC 49418^T	*Flavobacterium psychrophilum*		13/9	3.06 ± 0.16	4.22 ± 0.13
US019	*F. psychrophilum*		13/9	0.70 ± 0.00	3.40 ± 0.00
CSF259-93	*F. psychrophilum*		10/10	1.18 ± 0.03	2.66 ± 0.24
US305	*F. psychrophilum*		10/10	1.71 ± 0.19	4.05 ± 0.25
US075	*F. psychrophilum*		10/10	2.40 ± 0.40	4.54 ± 0.06
US051	*F. psychrophilum*		78/10	0.76 ± 0.06	4.57 ± 0.27
US053	*F. psychrophilum*		78/10	1.76 ± 0.24	3.75 ± 0.15
US074	*F. psychrophilum*		86/10	1.24 ± 0.24	3.18 ± 0.00
US073	*F. psychrophilum*		86/10	1.40 ± 0.30	3.55 ± 0.15
US104	*F. psychrophilum*		275/10	1.64 ± 0.20	4.85 ± 0.15
US057	*F. psychrophilum*		275/10	2.90 ± 0.00	8.54 ± 0.06[a]
US047	*F. psychrophilum*		256/256	3.18 ± 0.30	5.27 ± 0.13
US217	*F. psychrophilum*		256/256	1.09 ± 0.09	2.70 ± 0.00
US462	*F. psychrophilum*		286/286	3.20 ± 0.20	4.33 ± 0.15
US343	*F. psychrophilum*		301/191	0.36 ± 0.06	3.24 ± 0.24
US181	*F. psychrophilum*		301/191	0.17 ± 0.13	1.86 ± 0.08
US374	*F. psychrophilum*		330/318	1.70 ± 0.00	4.00 ± 1.00
US009	*F. psychrophilum*		253/singleton	3.55 ± 0.15	4.94 ± 0.24
US094	*F. psychrophilum*		253/singleton	4.09 ± 0.09	9.07 ± 0.16[a]
US442	*F. psychrophilum*		350/singleton	3.50 ± 0.20	4.90 ± 0.10
US443	*F. psychrophilum*		350/singleton	8.18 ± 0.03[a]	8.23 ± 0.00[a]
US450	*F. psychrophilum*		353/singleton	2.88 ± 0.18	5.03 ± 0.03
US451	*F. psychrophilum*		353/singleton	3.15 ± 0.33	3.86 ± 0.24
NIFA-508	*Flavobacterium psychroterrae*	96.6		3.91 ± 0.39	5.15 ± 0.15
ATCC BAA-2541^T	*Flavobacterium tructae*			2.70 ± 0.00	3.00 ± 0.00
NIFA-048	*F. tructae*	98.6		2.94 ± 0.24	4.09 ± 0.39
NIFA-037	*F. tructae*	98.7		0.85 ± 0.45	2.76 ± 0.06
NIFA-028	*F. tructae*	100		3.09 ± 0.09	3.91 ± 0.09
NIFA-147	*F. tructae*	98.8		3.54 ± 0.06	4.12 ± 0.42
ATCC 29473^T	*Yersinia ruckeri*			3.52 ± 0.00	4.78 ± 0.11

[a]Isolate was reduced by 100%.

[b]Columnaris-causing bacteria.

10^8–10^{10} colony-forming units (CFU)/mL for most (56/68 ≈ 82.4%) isolates. For the remaining 12 isolates, an identical OD_{600} yielded approximately 10^5–10^7 CFU/mL.

Exposure of bacteria to ultraviolet light

The collimating beam apparatus used in this study was supplied by AquiSense Technologies and consisted of a UVinaire single-wavelength (255-nm) UV LED unit and a collimating tube. The UVinaire was positioned on top of the collimating tube; when powered, the UVinaire produced an average UV intensity of 59.8 μW/cm^2 at the tube's end according to the manufacturer's specifications. The average UV intensity was used to calculate a target UV dose, which was the product of the average UV intensity and exposure time (s). Thus, by varying exposure time, different UV doses were achieved (Bolton and Linden 2003).

For this study, UV treatment doses of 25 and 126 mJ/cm^2 were evaluated for their ability to reduce bacterial concentration using the planktonic bacterial suspensions detailed in the previous section (Bacterial culture for ultraviolet light susceptibility experiments). For both UV treatment doses, 3 mL of each bacterial suspension were aliquoted into two

sterile 60-×15-mm petri dishes. Both petri dishes were placed on top of an orbital rotation platform that was set to slowly rotate at 60 rpm. One of the petri dishes on the orbital platform was underneath the collimating beam apparatus, while the other was not positioned under the apparatus and thus served as the negative control dish. The UVinaire was powered on for a duration equating to the evaluated UV doses. After treatment, the contents of both petri dishes were transferred into different sterile tubes and gently homogenized using a vortexer; bacteria were then quantified as described in the previous section.

Data analysis

Ultraviolet light efficacy was evaluated by calculating the $\log_{10}$ reduction in CFU, whereby the $\log_{10}$ was taken after dividing the number of CFU for the negative control group by the number of CFU for the treatment group.

A general linear mixed-effects model was used to quantify the effect (e.g., $\log_{10}$ reduction in CFU) of UV light treatment on the 65 flavobacterial isolates. The model included UV treatment dose, flavobacterial isolate group (i.e., columnaris-causing bacteria: $n = 7$; *F. psychrophilum* isolates: $n = 23$; all other flavobacterial isolates: $n = 35$), and the interaction between treatment dose and flavobacterial isolate group as fixed effects. Flavobacterial isolates within flavobacterial isolate group and the interaction between treatment dose and flavobacterial isolates within flavobacterial isolate group were treated as random effects. We treated flavobacterial isolates within flavobacterial isolate group as a random effect to draw inference for flavobacterial isolate variability beyond the flavobacterial isolates that were specifically measured for this study. Custom hypothesis tests examining the differences between the *F. psychrophilum* STs ($n = 12$) and between the columnaris-causing bacterial species ($n = 4$) at the same dose were evaluated through linear functions of model parameter estimates. Analyses were performed using the GLIMMIX procedure in SAS version 9.4; the construction of the custom hypotheses was performed using customized Contrast statements.

RESULTS

Ultraviolet inactivation of flavobacteria

General linear model analyses

Based on the fitted general linear mixed-effects model, the UV treatment doses had similar effects within each flavobacterial isolate group (i.e., there was no significant interaction between treatment dose and flavobacterial isolate group; $F = 0.80$; df = 2, 62; $p = 0.4550$); overall, flavobacterial isolate reduction was significantly greater at the high UV dose than at the low UV dose ($F = 73.17$; df = 1, 62; $p < 0.0001$; Figure 1A). In terms of the model random effects, variation was greater for the random effect of flavobacterial isolates within flavobacterial isolate group (variance = 2.886; SE = 0.6188) than for the interaction between treatment dose and flavobacterial isolates within flavobacterial isolate group (variance = 0.995; SE = 0.187).

Effect of low ultraviolet dose (25 mJ/cm^2)

At the low UV dose of 25 mJ/cm^2, the $\log_{10}$ reduction among all tested flavobacterial isolates ranged from 0.17 to 9.00 (Figure 1A), with 6 of the 65 evaluated flavobacterial isolates being reduced by 100% (Table 1). The $\log_{10}$ reduction for the columnaris-causing bacteria group, the *F. psychrophilum* isolate group, and the group containing all other flavobacterial isolates averaged 4.43 (SE = 0.749; range = 1.37–7.38), 2.34 (SE = 0.413; range = 0.17–8.17), and 3.95 (SE = 0.335; range = 0.84–9.00), respectively (Table 1; Figure 1B–D). The $\log_{10}$ reduction in the *F. psychrophilum* isolate group was significantly greater than the $\log_{10}$ reduction in both the columnaris-causing bacteria group ($t = 2.44$; df = 80.51; $p = 0.0168$) and the group consisting of all other flavobacterial isolates ($t = 3.03$; df = 80.51; $p = 0.0033$). The difference between the columnaris-causing bacteria group and the group containing all other flavobacterial isolates was not significant ($t = 0.58$; df = 80.51; $p = 0.5609$).

When columnaris-causing bacteria were grouped according to species, the average $\log_{10}$ reductions in bacterial concentration were 1.37 (*F. davisii*), 3.27 (*F. oreochromis*), 4.58 (*F. columnare*), and 6.96 (*F. covae*; Table 2). The $\log_{10}$ reductions for all species were significantly different from each other ($p < 0.0001$; Table S2).

When *F. psychrophilum* isolates were grouped by ST, the average $\log_{10}$ reduction ranged from 0.27 (ST301) to 5.84 (ST350; Table 2) and significant differences in UV light susceptibility were observed between all STs (Table S3). Sequence type 301 was significantly more resistant to UV light compared to all other STs ($p < 0.0001$), whereas ST350 was significantly less resistant to UV light in comparison to all other STs ($p < 0.0001$). The remaining 10 STs differed significantly in UV light susceptibility relative to 6 (ST330), 7 (ST256), 8 (ST10, ST13), 9 (ST78, ST86), 10 (ST256, ST275, ST353), or 11 (ST253) other STs (Table S3).

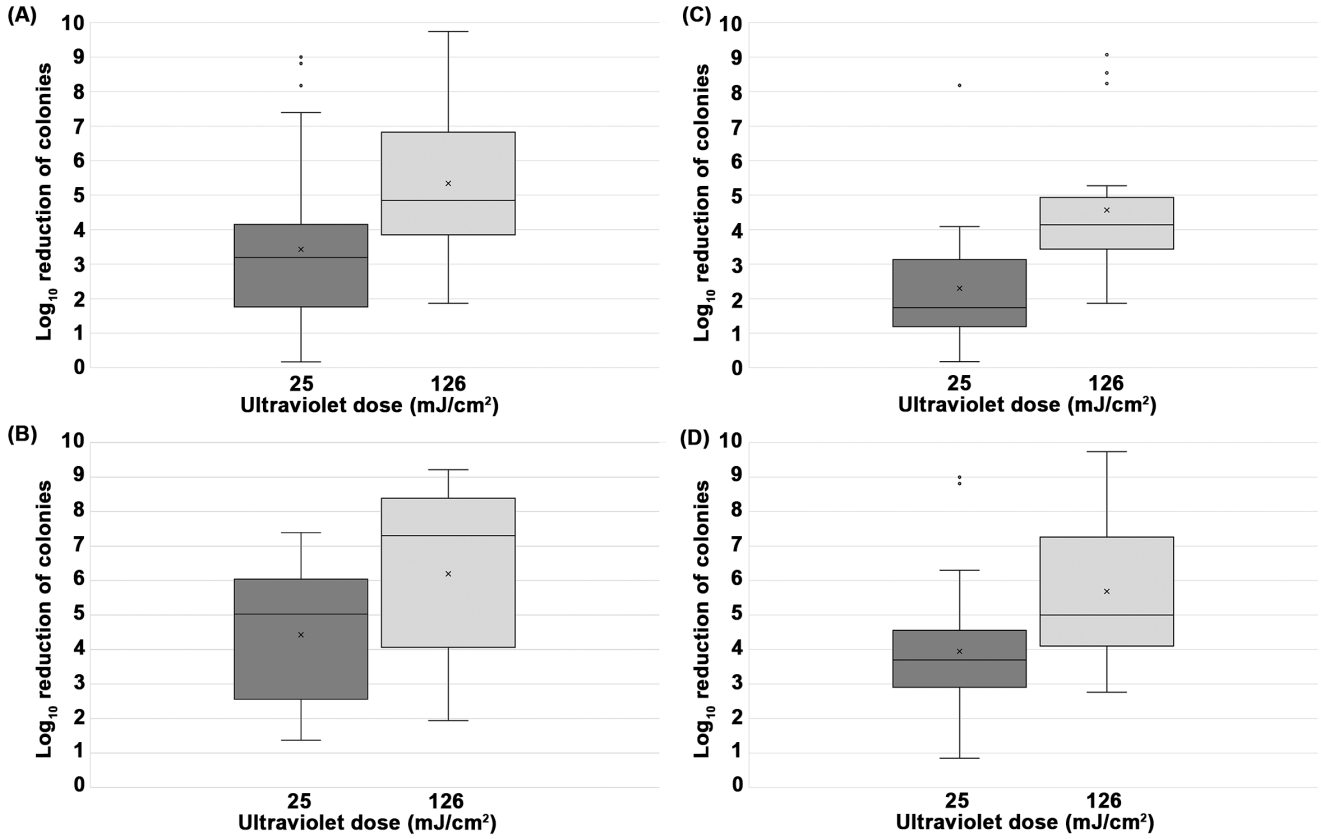

FIGURE 1 Box plots of the $\log_{10}$ reduction of colony-forming units at ultraviolet (UV) doses of 25 and 126 mJ/cm^2 for (A) all 65 flavobacteria isolates, (B) columnaris-causing bacteria ($n = 7$) only, (C) *Flavobacterium psychrophilum* isolates ($n = 23$) only, and (D) all 35 flavobacteria isolates except columnaris-causing bacteria and *F. psychrophilum*. All groups (A–D) were significantly more susceptible to UV light at the high dose compared to the low dose ($\alpha = 0.05$). Box plots depict the upper and lower quartiles, separated by the median (i.e., the horizontal line). Also included are the mean ("×" within the box) and the outliers (circles beyond whiskers).

Effect of high ultraviolet dose (126 mJ/cm^2)

At the high UV dose of 126 mJ/cm^2, the $\log_{10}$ reduction among all tested flavobacterial isolates ranged from 1.86 to 9.73 (Figure 1A), with 19 of the 65 evaluated flavobacteria isolates reduced by 100% (Table 1). The $\log_{10}$ reduction for the columnaris-causing bacteria group, the *F. psychrophilum* isolate group, and the group encompassing all other flavobacterial isolates averaged 6.20 (SE = 0.749; range = 1.93–9.21), 4.56 (SE = 0.413; range = 1.86–9.06), and 5.69 (SE = 0.335; range = 2.76–9.73), respectively (Table 1; Figure 1B–D). The $\log_{10}$ reduction in the group containing all other flavobacterial isolates was significantly greater than the reduction for the *F. psychrophilum* isolate group ($t = 2.13$; df = 80.51; $p = 0.0362$). The difference between the columnaris-causing bacteria group and the *F. psychrophilum* isolate group was not significant ($t = 1.92$; df = 80.51; $p = 0.0584$). The difference between the columnaris-causing bacteria group and the group consisting of all other flavobacterial isolates also was not significant ($t = 0.62$; df = 80.51; $p = 0.5364$).

When columnaris-causing bacteria were grouped according to species, the average $\log_{10}$ reductions in bacterial concentration were 3.85 (*F. davisii*), 5.36 (*F. oreochromis*), 6.75 (*F. columnare*), and 7.65 (*F. covae*; Table 2). The $\log_{10}$ reductions for all species were significantly different from each other ($p < 0.0001$; Table S2).

When *F. psychrophilum* isolates were grouped by ST, the $\log_{10}$ reduction ranged from 2.55 (ST301) to 7.00 (ST253; Table 2) and significant differences in UV light susceptibility were observed between all STs (Table S3). Sequence type 301 was significantly more resistant to UV light compared to all other STs ($p < 0.0001$–0.0002). Although ST253 had the greatest $\log_{10}$ reduction, it was not significantly different from those of two other STs (ST275: $p = 0.1212$; ST350: $p = 0.0666$). The remaining 10 STs differed significantly in UV light susceptibility relative to 5 (ST78, ST330), 6 (ST256), 7 (ST13, ST286), 8 (ST10, ST353), 9 (ST275, ST350), or 11 (ST86) other STs (Table S3).

TABLE 2 Log_{10} reduction of colony-forming units (mean ± SE) at ultraviolet doses of 25 and 126 mJ/cm^2 for four columnaris-causing bacterial species (e.g., *Flavobacterium columnare*, *F. covae*, *F. davisii*, and *F. oreochromis*) and 12 *F. psychrophilum* sequence types (STs), which belong to six clonal complexes (CCs) or are singletons. Columnaris-causing bacteria are presented first (alphabetically), followed by *F. psychrophilum* STs/CCs.

		Log_{10} reduction ± SE	
Species	**ST/CC**	**25 mJ/cm^2**	**126 mJ/cm^2**
F. columnare		4.58 ± 0.56	6.75 ± 1.42
F. covae		6.96 ± 0.27	7.65 ± 0.47
F. davisii		1.37 ± 0.15	3.85 ± 0.15
F. oreochromis		3.27 ± 1.02	5.36 ± 1.98
F. psychrophilum	13/9	1.88 ± 0.69	3.81 ± 0.24
F. psychrophilum	10/10	1.76 ± 0.25	3.75 ± 0.37
F. psychrophilum	78/10	1.26 ± 0.31	4.16 ± 0.27
F. psychrophilum	86/10	1.32 ± 0.16	3.36 ± 0.12
F. psychrophilum	275/10	2.27 ± 0.37	6.69 ± 1.07
F. psychrophilum	256/256	2.13 ± 0.62	3.99 ± 0.75
F. psychrophilum	286/286	3.20 ± 0.20	4.33 ± 0.15
F. psychrophilum	301/191	0.27 ± 0.08	2.55 ± 0.41
F. psychrophilum	330/318	1.70 ± 0.00	3.50 ± 0.50
F. psychrophilum	253/singleton	3.82 ± 0.17	7.00 ± 1.20
F. psychrophilum	350/singleton	5.84 ± 1.35	6.56 ± 0.96
F. psychrophilum	353/singleton	3.01 ± 0.17	4.45 ± 0.35

Comparisons between low (25 mJ/cm^2) and high (126 mJ/cm^2) ultraviolet doses

For the columnaris-causing bacteria group, the log_{10} reduction in bacterial concentration was significantly greater at the high UV dose than at the low UV dose ($t = 3.24$; df = 62; $p = 0.0019$). Similarly, the log_{10} reduction was significantly greater at the high UV dose than at the low UV dose for the *F. psychrophilum* isolate group ($t = 7.36$; df = 62; $p < 0.0001$) and for the group comprising all other flavobacterial isolates ($t = 7.13$; df = 62; $p < 0.0001$).

Ultraviolet light inactivation of nonflavobacteria

At the low UV dose, *C. maltaromaticum* was least susceptible among the nonflavobacterial species tested, exhibiting a log_{10} reduction of 1.50 ± 0.10 (mean ± SE), followed by *A. salmonicida* subsp. *salmonicida* and *Y. ruckeri*, which were reduced by 3.15 ± 0.15 and 3.52 ± 0.00, respectively (Table 1). At the high UV dose, *C. maltaromaticum* and *A. salmonicida* subsp. *salmonicida* were reduced similarly, with log_{10} reductions of 3.39 ± 0.09 and 3.39 ± 0.24, respectively. Comparably, reduction of *Y. ruckeri* was approximately 1.0 log higher at 4.78 ± 0.11 (Table 1).

DISCUSSION

Ultraviolet light susceptibility experiments with 65 flavobacterial isolates belonging to over 10 species of *Flavobacterium* and *Chryseobacterium* revealed reductions for all assayed taxa by an average of about 1000-fold at 25 mJ/cm^2 or by about 100,000-fold at 126 mJ/cm^2. However, some marked differences in UV light susceptibility between species and among isolates of the same species were observed. For example, at the UV dose of 25 mJ/cm^2, *F. psychrophilum* MLST variant ST301 was reduced significantly less (e.g., by less than twofold) than all other assayed MLST variants (reductions ranging from ~18-fold to 690,000-fold; Table 2), and the two most widespread, disease-causing *F. psychrophilum* variants in the United States (ST10 and ST78, both belonging to clonal complex ST10; Knupp et al. 2019) were among the most resistant to UV light (reductions ranging from ~18-fold to 57-fold; Table 2). Thus, it appears that UV light exposure could be more effective on some *F. psychrophilum* variants than others. Whether such variations in UV susceptibility explain in part the widespread, long-term persistence of *F. psychrophilum* variants ST10 and ST78 or others is currently unknown. Nevertheless, study findings revealed that UV light treatments have the potential to substantially reduce most *F. psychrophilum* isolates. Extrapolating

study results to facility source water, in which *F. psychrophilum* loads of approximately 10,000 cells/mL have been reported (Strepparava et al. 2014), while acknowledging that laboratory and field conditions (e.g., water turbidity) vary, a UV dose of 25 mJ/cm^2 could reduce many different *F. psychrophilum* isolates by 99%, thereby substantially reducing infection risk.

Findings for the four bacterial species that cause columnaris disease, which until recently was believed to be caused by only one species (*F. columnare*; LaFrentz et al. 2022), revealed that all species were reduced after UV light exposure at both doses. However, significant differences in reduction among the four newly described species were present. The factors driving these differences are unknown, but such factors are unlikely to include variations in cell morphology, as cell dimensions are similar among the four species (LaFrentz et al. 2022). Nevertheless, after future field studies are completed, it is possible that salmonid aquaculture facilities affected by *F. davisii* will need a higher UV dose than tilapia-producing facilities, which tend to be more affected by *F. oreochromis* (LaFrentz et al. 2022). Future studies evaluating the relationship between source water characteristics (e.g., turbidity) that vary among aquaculture facilities and the UV dose required to inactivate columnaris-causing bacteria and other flavobacteria under field conditions are warranted.

The mechanism or mechanisms responsible for the apparent reduced susceptibility of flavobacteria to UV light are currently unknown. However, research on flexirubin, a yellow pigment found at high concentrations in the outer membrane of some flavobacteria (Irschik and Reichenbach 1978; Venil et al. 2014), suggests that this pigment plays at least a partial role. In this context, Bai et al. (2017) mutated the flexirubin synthesis gene *fabZ* of *Cytophaga hutchinsonii* and found that nonpigmented mutants had reduced survival when exposed to UV light in comparison with the pigmented wild-type strain. Likewise, Venil et al. (2014) found that flexirubin isolated from a *Chryseobacterium* sp. was stable after 5 days of UV light exposure. Notably, *F. psychrophilum* isolates US181 and US343, the flavobacteria that were the least sensitive to UV light in this study, had the most intense and brightest yellow coloration compared to all other utilized flavobacteria (data not shown); however, a correlation between pigment intensity and UV resistance was not assessed herein and has yet to be described in flavobacteria elsewhere.

Although this study established a baseline UV light susceptibility profile for flavobacteria in a planktonic form, additional studies evaluating UV light efficacy against flavobacteria originating from biofilms are needed. Biofilm has been shown to protect other bacterial species, such as *Escherichia coli*, from the harmful effects of UV light (Vollmerhausen et al. 2017), likely by increasing the optical path to cells, light scattering by accumulated solids, and bacterial production of UV-absorbing pigments (Luo et al. 2022). Indeed, flavobacteria are also adept at forming biofilm on surfaces common to aquaculture and hatchery facilities (Cai et al. 2013; Levipan and Avendano-Herrera 2017; Sato et al. 2021). Likewise, at least one *Flavobacterium* sp. (i.e., *F. johnsoniae*) can form biofilm-like microcolonies on solid surfaces (Li et al. 2021). In this context, if flavobacteria in biofilm or biofilm-like assemblages are more resistant to UV light than flavobacteria in planktonic form, then higher UV doses may be required for inactivation.

Another area for future consideration comprises the mutational and therefore potential phenotypic effect(s) that UV light may have on different flavobacterial species, as UV light has been reported to induce recombination in some bacteria (Howard-Flanders et al. 1968). In this context, *F. psychrophilum* is highly recombinant according to MLST and whole-genome analyses (Duchaud et al. 2018; Knupp et al. 2019). Whether exposure of flavobacteria to UV light could ultimately lead to unanticipated phenotypic changes is currently unknown but warrants consideration.

In comparison to other bacterial fish pathogens that have been the subject of UV light efficacy studies, the flavobacteria evaluated herein appear more resilient to UV light exposure. For example, Liltved and LandFald (1996) exposed *Vibrio anguillarum*, *V. salmonicida*, and *Y. ruckeri* to a UV dose of 2.7 mJ/cm^2 and achieved an approximately 100,000-fold reduction for all three species. Similarly, a UV dose of 4–5 mJ/cm^2 was sufficient for reducing *Aeromonas hydrophila* and *A. salmonicida* subsp. *salmonicida* by about 1000-fold (Wedemeyer 1996). A similar overall degree of reduction for flavobacteria, as determined herein, was achieved at a UV dose of 126 or 25 mJ/cm^2, but an increase in UV dose did not result in a proportional increase in reduction for most flavobacterial isolates. Thus, whether UV doses lower than 25 mJ/cm^2 are also sufficient for reduction of flavobacteria remains unknown. Interestingly, although the *Y. ruckeri* (causative agent of enteric redmouth disease; Ross et al. 1966) and *A. salmonicida* subsp. *salmonicida* (etiological agent of furunculosis; Griffin et al. 1953) isolates evaluated in this study were reduced similarly to flavobacteria at the low UV dose (i.e., each by ~1000-fold), both bacterial isolates appeared less susceptible to UV light than did flavobacteria at the high UV dose (reduction of ~10,000-fold [*Y. ruckeri*] or ~1000-fold [*A. salmonicida* subsp. *salmonicida*] versus reduction of ~100,000-fold [flavobacteria]). Such discrepancies and the possible factor(s) behind them (e.g., methodological/technological differences, potential intraspecific variation in isolate UV susceptibility) warrant further study, but we strongly recommend that future UV light efficacy studies test multiple isolates of the same bacterial species.

Although not a primary goal of this study, the UV light susceptibility of *C. maltaromaticum*, the cause of pseudokidney disease in salmonids (Ross and Toth 1974), was evaluated herein for the first time. This bacterium appeared to be fairly resistant to UV light at both doses relative to the other studied isolates, which may not be surprising given that gram-positive bacteria are generally considered more UV light resistant than gram-negative bacteria due to differences in bacterial membrane structures (Mahapatra et al. 2007; Beauchamp and Lacroix 2012). Nevertheless, *C. maltaromaticum* may be an emerging fish pathogen that is also present in source water (Standish et al. 2022), and UV light appears to be a potential tool of use for its reduction.

In conclusion, UV light appears to be a promising means of reducing flavobacterial disease risk in fish farms and hatcheries. Although additional studies that more closely mimic the fish farming environment are needed, current results suggest that facilities afflicted by BCWD or *F. branchiophilum*-induced bacterial gill disease may benefit from treating the source water at a UV dose of 25 mJ/cm^2, which could result in a 99% reduction of viable cells, whereas facilities grappling with *F. davisii*-induced columnaris disease could consider implementing a UV dose of 126 mJ/cm^2 to achieve a similar reduction. Overall, the data produced herein are currently the most comprehensive source of information with respect to the UV light susceptibility of flavobacteria and will be beneficial for aquaculture and hatchery facility personnel in the interim.

ACKNOWLEDGMENTS

We thank the U.S. Department of Agriculture's National Institute of Food and Agriculture (Grants 2016-70007-25756 and 2019-70007-30417) for funding this research. We also thank past and present members of Michigan State University's Aquatic Animal Health Laboratory for their technical assistance in bacterial isolate recovery and preservation. We also appreciate the financial support provided by Michigan State University AgBioResearch to T.L. through the Stanislaus F. Snieszko Endowed Scholar program.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

No ethical guidelines were applicable, as no live fish were used in this study.

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