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Supporting Information

for

Antiviral therapy in shrimp through plant virus VLP containing VP28 dsRNA against WSSV

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Tables of detailed experimental assays and methods to prepare the pellet feed containing VLP-dsRNAvp28

Table ST1. Partial sequence of the VP28 gene of the main structural protein of WSSV isolated from Mexico (GenBank: EU931451.1).

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ATGGATCTTTCTTTCACTCTTTCGGTCGTGTCGGCCATCCTCGCCATCACTGCTGTGATTGCTGTATTTA
TTGTGATTTTTAGGTATCACAACACTGTGACCAAGACCATCGAAACCCACACAGACAATATCGAGACAAA
CATGGATGAAAACCTCCGCATTCCTGTGACTGCTGAGGTTGGATCAGGCTACTTCAAGATGACTGATGTG
TCCTTTGACAGCGACACCTTGGGCAAAATCAAGATCCGCAATGGAAAGTCTGATGCACAGATGAAGGAAG
AAGATGCGGATCTTGTCATCACTCCCGTGGAGGGCCGAGCACTCGAAGTGACTGTGGGGCAGAATCTCAC
CTTTGAGGGAACATTCAAGGTGTGGAACAACACATCAAGAAAGATCAACATCACTGGTATGCAGATGGTG
CCAAAGATTAACCCATCAAAGGCCTTTGTCGGTAGCTCCAACACCTCCTCCTTCACCCCCGTCTCTATTG
ATGAGGATGAAGTTGGCACCTTTGTGTGTGGTACCACCTTTGGCGCACCAATTGCAGCTACCGCCGGTGG
AAA
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Table ST2. Design of the challenge to evaluate the efficacy and optimal dose of dsRNA*vp28*.

Group[1]	**Treatment scheme**	**No. of shrimp**[2]
WSSV-Positive	+WSSV infected shrimp	4 × 5
3.0 µg WSSV-Negative	-WSSV free shrimp 3.0 µg dsRNA*vp28* IM	4 × 5
0.5 µg dsRNA*vp28*	+0.5 µg dsRNA*vp28* IM	4 × 5
1.0 µg dsRNA*vp28*	+1.0 µg dsRNA*vp28* IM	4 × 5
2.0 µg dsRNA*vp28*	+2.0 µg dsRNA*vp28* IM	4 × 5
3.0 µg dsRNA*vp28*	+3.0 µg dsRNA*vp28* IM	4 × 5

[1]Indicates the abbreviature for each treatment
(+) WSSV-Positive shrimp with a dose of 10^{-6}; (-) WSSV-Negative or non-infected shrimp. [2]Indicates the number of replicates × number of shrimps in each replicate.

Methods to prepare the pellet feed containing VLP-dsRNAvp28.

Method I. Pellets of similar size (Natural Force, VIMIFOS. México) were coated with 0.5 µg of VLP-dsRNAvp28 each. VLP-dsRNAvp28 were applied coating the pellets with a thin layer of fish oil before drying at room temperature [1]. The feed was dried at 28 °C for 15 min and stored at 4 °C. The pellets were offered twice a day (2.0 µg/shrimp/day), 2 pellets at 09:00 h. and two at 16:00 h.) for three continuous days. After 48 hour feeding period, the shrimp were challenged with the WSSV.

Method II. The pellets were pulverized using a sterile porcelain mortar until a fine powder was obtained and mixed with 3% (w/v) NutriKelp® binder and fish oil in hot water (90 °C). After the blend was cooled to ca. 35 ° C, the solution of VLP-dsRNAvp28 and 5% fish oil was added. The proportion recipe for the preparation of the pellets is shown in Table ST3. Afterward, the mixture was pelleted pressing through a 50 mL syringe without a needle (Terumo® 50 mL) to make long pellets (≈ 10–12 cm) and dried in an incubator (VWR Incubator F Air 2.3 CF, USA) at 28 °C for 40 min. The pellets were cut into pieces of ≈ 2 to 2.5 mm long. Feed prepared with VLPs was stored at 4 °C for subsequent doses. The pellets obtained (10 g) were weighed and divided by the number of organisms to be treated (n = 20). The ration for each shrimp (0.5 g) was divided into six portions, to offer twice a day. The feed was administered at 2 µg of dsRNAi/shrimp daily, as described in the Method I section, giving a total of 6 µg of dsRNA/shrimp over a period of three days. In both cases the organisms were starved for 24 hours [2] before giving them the VLP-dsRNAvp28 treatment.

Pellets with VLP-dsRNAvp28 coated with industrial grade fish oil. Industrial grade fish oil was used to coat the pellets with VLPs following the Method I and II. The treatments were performed using shrimp (10.0 g ± 1.1 g) acclimated for 15 days at a salinity of 16 ppt. The experimental conditions are detailed in Table ST4 (Experiment 1).

Pellet with VLP-dsRNAvp28 coated with salmon fish oil. The salmon fish oil (Carlson Labs, Wild Norwegian Cod Liver Oil) was used to coat the pellets with VLPs following the Method I and II. Seven treatments were performed, each with four replicates with four shrimp (14.5 g ± 2.1 g) each (n = 16) in 16 ppt saline water. The details are described in Table ST4, Experiment 2.

Pellet with VLP-dsRNAvp28 prepared with commercial binders.

a). Dry Oil®. The pellet feeds were prepared as described in Method I with slight changes. Dry Oil® (DO, Innova-Codemet SA de CV, Mexico) was used as a binder to coat pellets with VLPs, following the manufacturer's instructions. The pellets were directly submerged in a solution of Dry Oil in TN buffer at 30 ℃ containing the VLP-dsRNAvp28. The Table ST4 describe in details of this treatment, Experiment 3. Due to the characteristics of this product, it was only used to coat the pellets.

b). NutriKelp®. Pellets with VLPs using NutriKelp® (NK, NutriKelp Algas & Bioderivados Marinos, Mexico) as a binder. The procedure to make this food is described in "Method II." Experimental details are in Table ST4 (Experiment 3). The Nutrikelp has an alginate base; it was only used to remake the pellet and not to coat them.

Table ST3. Recipe to prepare pellets mixed with VLPdsRNA*vp28* to feed shrimps.

Ingredient	Amount	To observe
Powdered pellet	8.0 g/ Xg[a]	The formation of fine grains
Natural grenetine hydrated		3% total in the mix
Fish oil	0.5 mL	
VLPdsRNA*vp28** (0.12 µg/µL)	1.0 mL	1X PBS[b]
Pellet mixed with VLPdsRNA*vp28*	≈ 10 g	Make pellet

[a]The amount used may vary depending on the shrimp weight, to administrate twice a day, approximately 3% daily biomass for 3.3 g shrimp.
[b]Make pellet with 50 mL syringe without needle, and dry at 28–29 °C × 40 min, then cut in pieces 2 to 2.5 mm long.

Table ST4. Oral feeding challenges with VLPdsRNA*vp28* in *Penaeus vannamei*.

Experiment	**Group***	**Treatment scheme**	**No. of shrimp in group[a]**
Exp. 1.	WSSV-Positive-E1	+WSSV infected shrimp	4 × 5
	WSSV-Negative-E1	-WSSV free shrimp	4 × 5
	VLP28-IM-E1	+Control VLPdsRNA*vp28* IM	4 × 5
	ApVLP28-mix-E1	+VLPdsRNA*vp28*/Mixpell	4 × 5
	ApVLP28-coat-E1	+VLPdsRNA*vp28*/coatpell	4 × 5
	VLP28-oral cav-E1	+VLPdsRNA*vp28*/oral cavity	4 × 5
	dsRNA28-200 µg-IM	+Naked dsRNA*vp28*/200 µg IM	2 × 6
	dsRNA28- 6 µg IM-E1	+Naked dsRNA*vp28* -IM	4 × 5
Exp. 2.	WSSV-Positive-E2	+ WSSV infected shrimp	4 × 4
	WSSV-Negative-E2	-WSSV free shrimp	4 × 4

	VLP28-IM-E2	+Control VLPdsRNA*vp28* IM	4 × 4
	ApsVLP28-mix-E2	+VLPdsRNA*vp28*/Mixpell	4 × 4
	ApsVLP28-coat-E2	+VLPdsRNA*vp28*/coatpell	4 × 4
	VLP28-Oral cav-E2	+VLPdsRNA*vp28*/oral cavity	4 × 4
	dsRNA28-Oral cav-E2	+Naked dsRNA*vp28* /Oral cavity	4 × 4
	WSSV-PositiveE3	+WSSV infected shrimp	1 × 10
	WSSV-Negative-E3	-WSSV free shrimp	2 × 15
Exp. 3.	VLP28-IM-E3	+Control VLPdsRNA*vp28* IM	3 × 10
	NKVLP28-mix-E3	+VLPdsRNA*vp28*/Mixpell	3 × 10
	DOVLP28-coat-E3	+VLPdsRNA*vp28*/coatpell	3 × 10

*=Treatment key. E = Experiment number.
"+"=WSSV-Positive (dose 10^{-6}); "-" WSSV-Negative, Mixpell = mixed pellet, coatpell = coated pellet.
Shrimps treated with VLPdsRNAvp28 or naked dsRNAvp28 received a dose of 6 µg dsRNA, unless otherwise specified. [a]Indicates the number of replicates × number of shrimps in each replicate.

Exp. 1. Pellets with VLPdsRNA*vp28* prepared with fish oil (Ap) (industrial grade)

Exp. 2. Pellet with VLPdsRNA*vp28* coated with salmon fish oil (Aps)

Exp. 3. Pellet with VLPdsRNA*vp28* prepared with commercial binders (DO/NK= (Dry Oil® and NutriKelp®, respectively).

References

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2. Ning, J. F.; Zhu, W.; Xu, J. P.; Zheng, C. Y.; Meng, X. L. *Vaccine* **2009,** *27,* 1127–1135. doi:10.1016/j.vaccine.2008.11.075