Data in brief 25 (2019) 104221

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/dib

Data Article

The correlations data between whether liposomes are coated or not and flatfish's (*paralichthys olivaceus*) *Streptococcus parauberis* antibody formation ability and survival rates

Eun Sol Jo [a], Hyun-Ja Han [c], Deok Chan Lee [c], Kwang Il Kim [c], Mi Young Cho [c], Sung-Hee Jung [c], Chan-Il Park [d], Andre Kim [b, *]

[a] *Natural Science Institute, Silla University, Busan 46958, Republic of Korea*
[b] *Department of Pharmaceutical Engineering Division of Bioindustry, College of Medical and Life Science Silla University, Busan 46958, Republic of Korea*
[c] *Pathology Research Division, Aquaculture Research Department, National Institute of Fisheris Sicience, Busan 46083, Republic of Korea*
[d] *Institute of Marine Industry, College of Marine Science, Gyeongsang National University, 455, Tongyeong 650-160, Republic of Korea*

ARTICLE INFO

Article history:
Received 15 February 2019
Received in revised form 10 May 2019
Accepted 28 June 2019
Available online 5 July 2019

ABSTRACT

Streptococcus parauberis is a known etiologic agent that causes damage leading to death in flatfish (*paralichthys olivaceus*). Liposomes were used to deliver streptococcal oral vaccines to the intestinal mucous membranes of *paralichthys olivaceus*. The liposomes were coated for stabilization, and stability was measured with high performance liquid chromatography (HPLC). The liposomes were stable until day nine and were orally administered to flatfish as a vaccine. The resultant antibody titers were analyzed. The titers resulting from the uncoated liposomes were highest two weeks after the oral administration, and those resulting from the coated liposomes were highest one week after boosting. In addition, the bacteria were subcutaneously injected to artificially infect flatfish and the survival rates and relative survival rates were analyzed. The coated liposomes were found to yield the highest survival rate.

* Corresponding author.
E-mail address: adrk@silla.ac.kr (A. Kim).

https://doi.org/10.1016/j.dib.2019.104221

Specifications table

Subject area	*Fishery vaccine*
More specific subject area	*Liposome vaccine*
Type of data	*graph*
How data was acquired	*HPLC, ELISA reader*
Data format	*analyzed*
Experimental factors	*A coated, uncoated liposome containing Streptococcus parauberis was prepared.*
Experimental features	*Increased efficiency of vaccine*
Data source location	*Silla university, korea*
Data accessibility	*Data are available within this article*
Related research article	*R. Harikrishnan, J. S. Kim, C. Balasundaram, M. S. Heo, Vaccination effect of liposomes entrapped whole cell bacterial vaccine on immune response and disease protection in Epinephelus bruneus against Vibrio harveyi. Aquaculture, 2012. P. 342–343 [1]*

Value of the data

- The data can be used to show the immune stability of coated and uncoated liposomes.
- This data may be useful for studies of liposomes for the assessment of Paralichthys olivaceus streptococcal oral vaccines.
- This data presents a new method of development for the flounder streptococcal oral vaccine.

1. Data

FKC was used as an oral vaccine and FKC was obtained by adding formalin to the *Streptococcus parauberis* (KSP28). Liposomes were prepared by ultrasonic dispersion using a lecithin and cholesterol. The stability rates of coated and uncoated liposomes for 0, 3, 6, 9, 12, 15, 18, and 21 days were analyzed and the results are presented in Fig. 1. The coated liposomes were more stable than the uncoated liposomes by approximately 10% and the stability rates changed little from day 12. Therefore, the liposomes at day 12 were mixed with feed and orally administered to three groups of 30 flatfish on days 1, 3 and 5 respectively.

The oral administration was conducted by directly injecting the liquid phase oral vaccine solution into the gastric lumens using syringes. The experiment was conducted with a group administered coated liposomes containing FKC, a group administered uncoated liposomes containing FKC, a blank group receiving no treatment at all, a control group orally administered phosphate buffer saline (PBS) 100 μl mixed with feed, an Intraperitoneal (IP) injection group intraperitoneally injected with the bacteria at a concentration of 1 mg/ml, and an oral group orally administered the bacteria at a concentration of 3 mg/ml. The serums were separated two weeks after oral administration and the antibodies were analyzed. The results are presented in Fig. 2. The coated liposomes showed higher antibody titers compared to the control and the uncoated liposomes showed the highest antibody titers. Afterward, the antibody titers in the mucous membrane were separated from the bowel tissues one week after boosting and the serum separated from the blood were analyzed with the results shown in Fig. 3. In the serum, the antibody titer of coated liposomes was highest and the antibody titer of uncoated liposomes was shown to be similar to the blank titer. In the mucous membrane, the antibody titer of coated liposomes was the highest as well, followed by that of the uncoated liposomes.

The bacteria were subcutaneously injected at a concentration of 10^6 CFU/fish three weeks after oral administration and the survival rates and relative survival rates were analyzed for 15 days. The results are shown in Figs. 4 and 5. The coated liposomes showed higher survival rates than the uncoated liposomes.

2. Experimental design, materials and methods

2.1. Preparation of liposome oral vaccine containing FKC

Streptococcus parauberis (KSP28) was inoculated into BRAIN HEART INFUSION BROTH (BHIB) medium, incubated for 24 h, formalin added to reach a concentration of 0.5%, and left unattended at

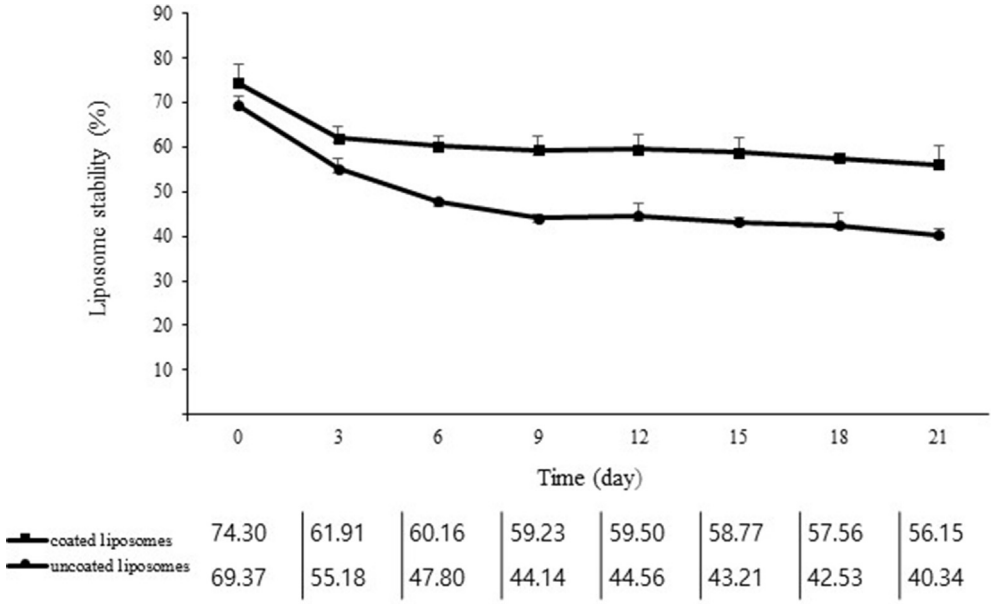


Fig. 1. Stability of FKC in liposomes during the storage period (coated and uncoated). The data represent mean ± standard deviation of three replicates from each sample.

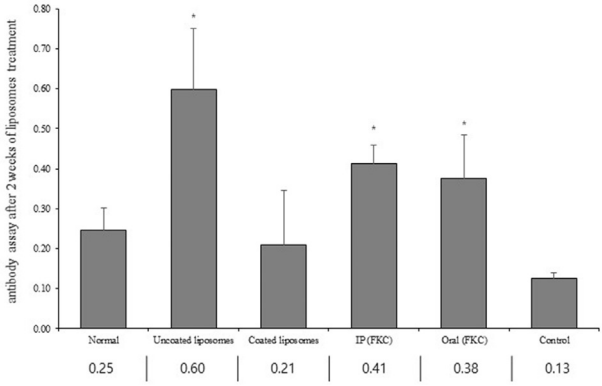


Fig. 2. Liposomes containing *S. parauberis* FKC were orally administered to flatfish at a concentration of 3 mg/fish. After 2 weeks, ELISA was used to analyze specific antibody formation ability. The data represents mean ± SD for triplicates ($p < 0.05$).

room temperature for 24 h. Pellets were obtained through centrifugation for 30 min at 7,000 rpm and washed with PBS three times to use with the *S. parauberis* FKC in the experiment (FKC). To obtain liposomes, 0.5 g of lecithin and 0.1 g of cholesterol were dissolved with chloroform and the solvent was evaporated in a 60 °C rotary evaporator to form thin lipid membranes. After adding 5 ml of FKC to the thin lipid membranes, ultrasonic waves were applied six times for 5 s each with a probe type sonicator to disperse the thin lipid membranes. After adding 10 ml of PBS buffer, the dispersed substances were centrifuged for 10 min at 3,000 rpm to obtain liposomes containing FKC. Chitosan coated liposomes containing FKC were obtained by mixing the substances with 0.5% chitosan and centrifuging the mixture [1].

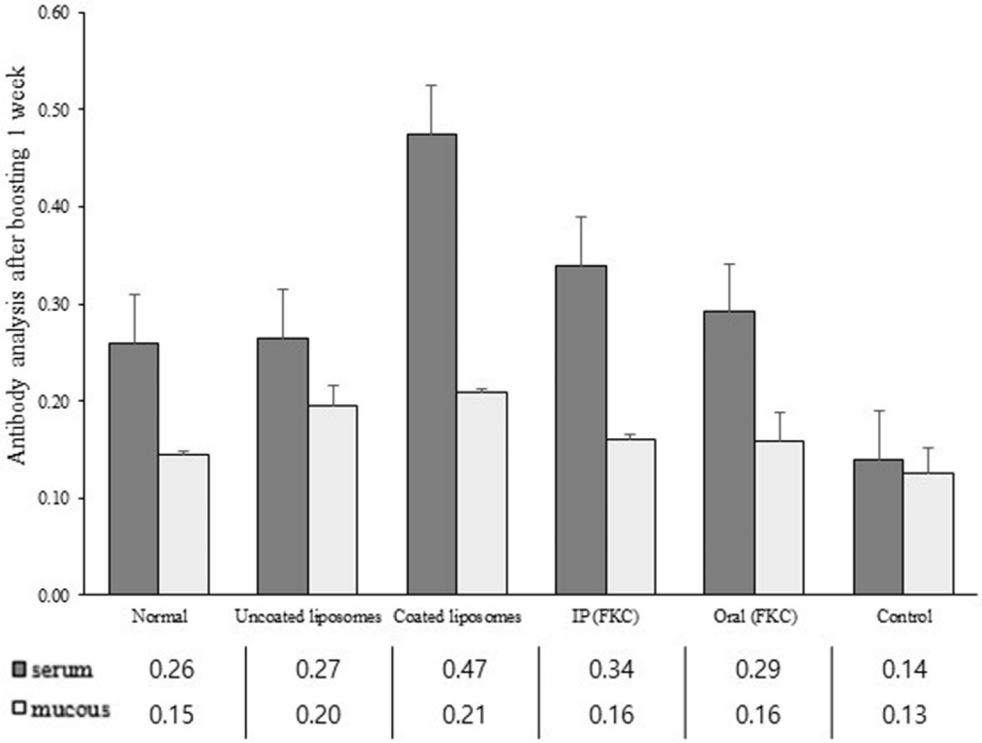


Fig. 3. Liposomes containing *S. parauberis* FKC were orally administered to flatfish at a concentration of 3 mg/fish. One week after boosting, the ability to form specific antibodies was analyzed by ELISA. The data represents mean ± SD for triplicates.

2.2. Liposome stability experiment

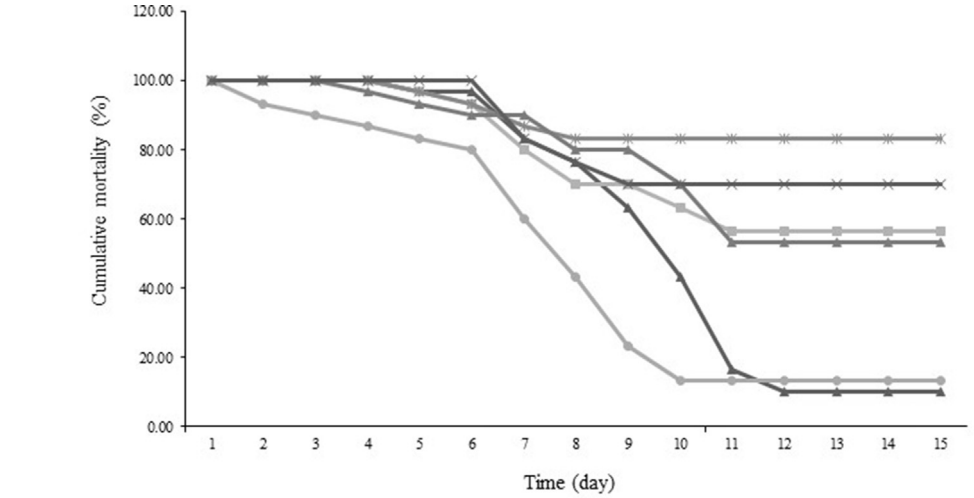
For the liposome stability experiments, liposomes containing gallic acid were prepared and kept for 21 days at an ambient temperature of 4 °C. To analyze liposome stability rates, the liposomes were centrifuged and the quantities of gallic acid in the upper layer and lower layer were analyzed every three days with HPLC (Alliance e2695 Separations Module, Korea). Using a C18 column (SunFire™, 4.6 x 300 mm, 5 μm) as a column and phosphate buffer (pH 6.8) and acetonitrile = 65:35 as an analysis solvent, the quantities of gallic acid were measured at UV 222 nm with a 10 μl quantity of the injected sample and a flow velocity of 1 ml/min. Ibuprofen was used as a standard. The stability rates were calculated according to the following calculation formula.

$$\text{Stability rate } (\%) = \frac{\text{A or B}}{A+B} \text{x} 100$$

A is the area ratio of gallic acid to ibuprofen in the upper layer and B is the area ratio of gallic acid to ibuprofen in the lower layer.

2.3. Analysis of the liposomes' ability to present and transport antigens and form immunity

The liposome oral vaccine containing FKC was mixed with feed powder and prepared in the liquid phase, after adjusting the concentration to a dose of 3 mg/fish. The oral administration was conducted by directly injecting the liquid phase oral vaccine solution into the gastric lumens using syringes.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Normal	100	100	100	100	96.67	96.67	83.33	76.67	63.33	43.33	16.67	10	10	10	10
Uncoated liposomes	100	100	100	100	96.67	93.33	80	70	70	63.33	56.67	56.67	56.67	56.67	56.67
Coated liposomes	100	00	100	100	96.67	93.33	86.67	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33
Control	100	93.33	90.00	86.67	83.33	80	60	43.33	23.33	13.33	13.33	13.33	13.33	13.33	13.33
Oral	100	100	100	96.67	93.33	90	90	80	80	70	53.33	53.33	53.33	53.33	53.33
IP	100	100	100	100	100	100	83.33	76.67	70	70	70	70	70	70	70

Fig. 4. Survival rates for artificial infection after administration of coated and uncoated liposomes containing *S. parauberis* FKC.

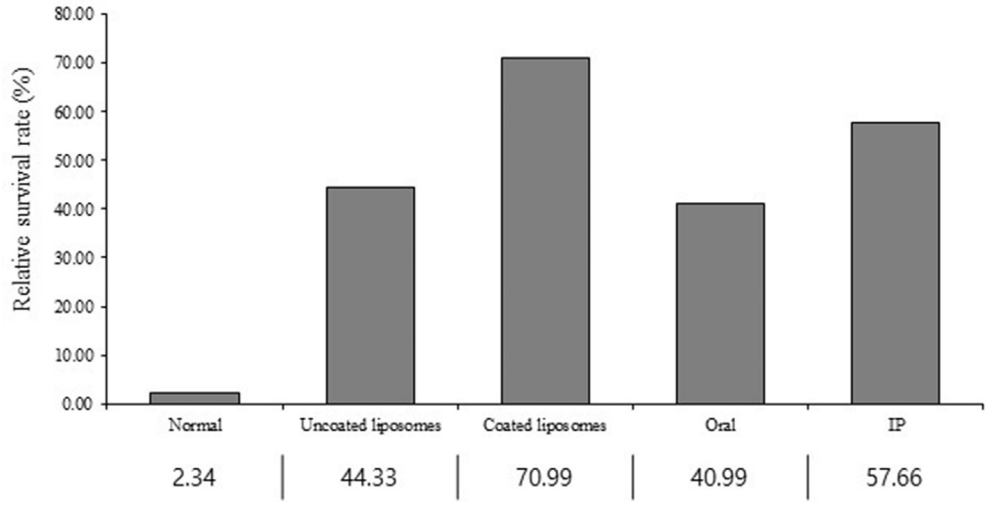


Fig. 5. Relative survival rate of flatfish treated with coated and uncoated liposomes containing *S. parauberis* FKC.

The liposome oral vaccine was administered to three groups of 30 flatfish on days 1, 3 and 5 respectively. The experiment was conducted with a group administered coated liposomes containing FKC, a group administered uncoated liposomes containing FKC, a blank group receiving no treatment at all, a

control group orally administered PBS 100 μl mixed with feed, an IP group intraperitoneally injected with the bacteria at a concentration of 1 mg/ml, and an oral group orally administered with the bacteria at a concentration of 3 mg/ml. Two weeks later, for antibody titer analysis, peripheral blood was extracted from the tail vein, left unattended for 1 h at 4 °C and centrifuged for 15 min at 6,000 rpm to separate the serum. One week after boosting, the blood was extracted using the same method and the serum was separated. The bowel was extracted through dissection, the foreign matter was removed using PBS, and the mucous membrane was separated by softly scratching the inside of the bowel using a spatula. Thereafter, 1.5 ml of PBS was added to the mucous membrane, and the mucous membrane was passed through a 0.45 μm syringe filter and used in the antibody titer analysis. The antibody titer analysis was conducted as explained in [2]. To analyze survival rates and relative survival rates, coated and uncoated liposomes were orally administered, the antigen was intraperitoneally administered, and the bacteria were subcutaneously injected at a concentration of 10^6 CFU/fish for artificial infection. Thereafter, the survival rates and relative survival rates were analyzed for 15 days [3].

Acknowledgements

This work was supported by the National Institute of Fisheries Sciences(NIFS R2018063).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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