The JSFS 85th Anniversary-Commemorative International Symposium
"Fisheries Science for Future Generations"
Symposium Proceedings, No. 02014

Original article

Effective gene delivery of chitosan nanoparticles in giant freshwater prawn (*Macrobrachium rosenbergii*)

Pichai Phongudom [1], Suwalee Ma-anan [1], Chuphong Phakpum [2], Chalermchail Pilapong [3] and Jiraporn Rojtinnakorn [1,*]

[1] *Faculty of Fisheries Technology and Aquatic Resources, Maejo University, San Sai, Chiang Mai, 50290, Thailand*
[2] *Faculty of Science, Maejo University, San Sai, Chiang Mai, 50290, Thailand*
[3] *Faculty of Associated Medical Sciences, Chiang Mai University, Muang, Chiang Mai, 50200, Thailand*
* Correspondence: jiraroj@mju.c.th; Tel.: +66-5-3-8751-00

Keywords: Chitosan nanoparticles, Gene expression, Toxicity, Histopathology, Giant freshwater prawn.

Received: 18 July 2017 / Accepted: 16 September 2017

Introduction

Chitosan nanoparticles (CS-NPs) are solid colloidal particles which have special properties such as biocompatibility, biodegradability and mucosal delivery activity [1]. In human, CS-NPs has been applied as carrier, adhesive and active molecules to target organs, and to enhance the performance of drugs. Innovative functional foods that can bring physiological benefits or reduce long-term risks of developing diseases which are being developed by the scientific community [2]. There were few reports of CS-NPs available relevant to fish and shrimp. Moreover, there is no any research involving toxicity of CS-NPs in aquatic animals.

In this study, chitosan nanoparticles were synthesized by ionotropic gelation technique using commercial chitosan and tripolyphosphate (TPP) as crosslinker.

Materials and methods

Materials
Chitosan % DD = 81.45 (TMECO), sodium tripolyphosphate 95% purity (Bio basic) and glacial acetic acid (Merck).

Preparation of chitosan nanoparticle
CS-NPs were prepared with ionotropic gelation technique [3] using chitosan in 1% (w/v) acetic acid and sodium tripolyphosphate as crosslinker. Nanoparticles were collected, freeze dried and stored at 4°C until using.

Characterization of Chitosan Nanoparticles
The morphologic characterization of chitosan nanoparticles was evaluated by using a scanning electron microscopy (SEM) (JSM-5410 LV; JEOL, Japan). The particle size and zeta potential were determined using nanosizer (S4700; Malvern, English).

Food preparation coated with chitosan nanoparticles
Chitosan nanoparticles as a dry powder to be dissolved in water in a ratio of 1:1 (w/v). Shrimp food number 2, Sprayed with dissolved nanoparticles in a ratio of 0.1, 0.5 1.0 and 5.0% (w/w) all over, then desiccate and store food in a closed container.

In vivo toxicity test
Tissue of representative giant freshwater prawns from each test and control group was tested. Subsequently, the organs including gill, liver, pancreas, stomach intestines and heart were prepared. Fixative samples in 95% ethanol for 24 hours, then stored at 75% ethanol, were performed section cutting and hematoxylin–eosin stain, consider the differences with the control group. Before being viewed under a light microscope [4].

In vivo gene expression test
The GFP gene into an expression plasmid (expression vector pcDNA3.1 eukaryotic), the GFP plasmid are to be transferred into *Escherichia coli* (DH5α) cells. To increase the number of plasmids (pGFP). Plasmid extraction from *E. coli* cells were used for further experiments. Prepared encapsulated chitosan nanoparticles were containing pGFP (CS-NPs- pGFP). Giant freshwater prawns were fed with 0.1% (w/w) CS-NPs-pGFP (1 μg per prawn); and followed the expression of fluorescent proteins.

Results

Physical and chemical characteristics of nanoparticles were identified by SEM, nano-sizing and zeta potential. It indicated that CS-NPs had size of 274±73 nm with zeta potential range of -11.16±0.467 mV.

For *in vivo* toxicity test, giant freshwater prawn was fed with CS-NPs 0.1, 0.5, 1 and 5% (w/w) added feed.

Histopathology of interested tissues, i.e. heart, gill, stomach, intestine, liver and pancreas studies were followed at 2 and 4 weeks of feeding. It showed that heart, gill, stomach; and intestine of groups showed the normal cells and distinctly structure. Liver and pancreas tissues of all high dose groups showed the abnormal cells in liver tissue, hepatopancreatic tubularis separating from reserve inclusion cells were observed. Whereas in low dose group, all tissue was normal.

For *in vivo* gene expression test, pGFP encapsulated in chitosan nanoparticles (CS-NPs-pGFP) were used. Giant freshwater prawn was fed with CS-NPs-pGFP 0.1 % (w/w) (1 µg per prawn) for "one day". The expression was analyzed under the fluorescent microscope at 1, 3, 5 and 7 days after feeding, it showed that green fluorescence in all organs such as gill, stomach, intestine, heart, brain and pancreas beginning at 1 day after feeding and increasing till 7 days (Fig. 1).

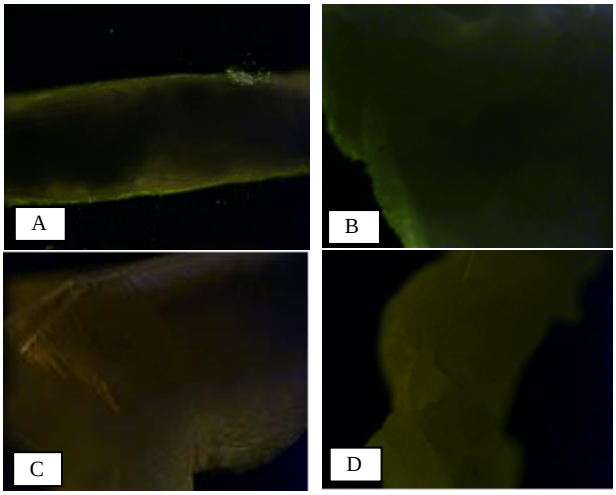

Fig. 1. It showed green fluorescence in all organs such as intestine (A), heart (B), gill (C) and stomach (D) at 3rd day after feeding.

Discussion

Physical examination with SEM, demonstrates the particle size of the nano-solar chitosan and the zeta potential with a nano-sizing. The size of chitosan nanoparticles that was small, have a rounded shape. Nanoparticles visible that colloid translucent of the gel is clear. When tested with SEM found that chitosan particles of the shape are round, and small [5].

The toxicity of chitosan nanoparticles was found in muscle tissue; and the liver and pancreas are dysfunctional. Compared to the control group, abnormal occurred in the development of mucus cells (MC) and epithelial cells (EPC) which has intermittent spaces. Tissue normal of shrimp muscle it consists of long-form cells in parallel which is attached to the fiber, development of MC and EPC appear together clearly There are alternately stretch and shrink according to the curvature of the body [6].

The organ of expression was CS-NPs-pGFP to be an organ about the digestive system and the excretory system of waste. Also found in the most luminous organs, liver, kidneys, stomach and intestines which appears in the light green fluorescent protein. There are reports to the use of chitosan nanoparticles for delivery of DNA vaccines. Study found that DNA vaccines, can be delivered to the fish, use of chitosan nanoparticles for the delivery of DNA vaccines. By bringing chitosan nanoparticles, mix with food to feed for fish. Also showed gene expression on the liver, spleen and colon [7].

Finally, this study results, concluded that our chitosan nanoparticles well carried gene for tissue expression with low toxicity in shrimp. Thus, it can be continued available as gene delivery system by feed additive for shrimp and other aquatic animals.

Acknowledgements

This work was financially supported by Research and Researchers for Industries (RRi), the Thailand Research Fund (TRF). Also, we would like to thank to the Faculty of Fisheries Technology and Aquatic Resources, Maejo University for the funding to join the JSFS 85th Anniversary-Commemorative International Symposium.

References

1. Denkbas EB et al. (2002) React Funct Polym 50: 225–232
2. Elliott R, Ong TJ (2002) Br Med J 324: 1438–1442
3. Calvo P et al. (1997) Pharm Res 14: 1431–1436
4. Bell TA, Lightner DV (1988) A Handbook of Normal Penaeid Shrimp Histology, World Aquaculture Society, Baton Rouge, LA, pp. 1–9
5. Pan Y et al. (2002) Int J Pharm 249: 139–147
6. Sinchaipanit P (2012) Doctor of Philosophy Thesis Ph.D, Chulalongkorn University 254 pp
7. Kumar RS et al. (2007) Fish Shellfish Immunol 23: 16–26