DEVELOPMENT OF A NOVEL IMMERSION VACCINE AGAINST SCUTICOCILIATOSIS IN OLIVE FLOUNDER

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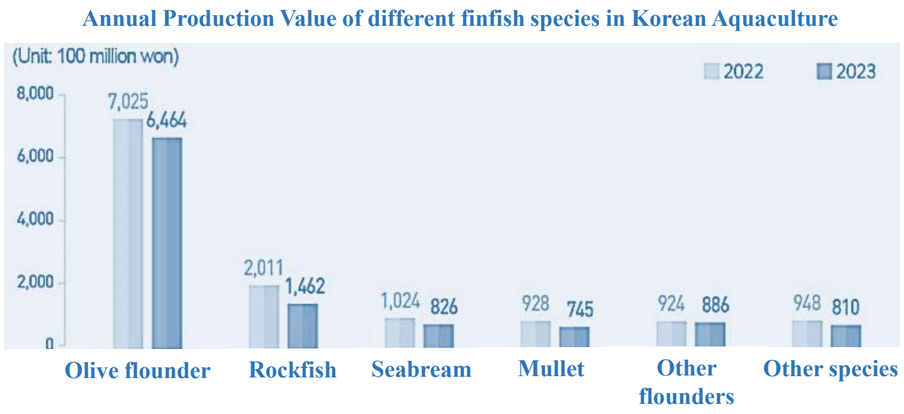
Introduction

➢ **Olive flounder (*Paralichthys olivaceus*) aquaculture in South Korea**

- **High economic fish species, high growth rate and feed efficiency**
- **Aquaculture started from 1980s**
- **Majorly land-based flow-through system (>50% in Jeju Island)**
- **Most produced finfish species in Korea, over 70% global supply**

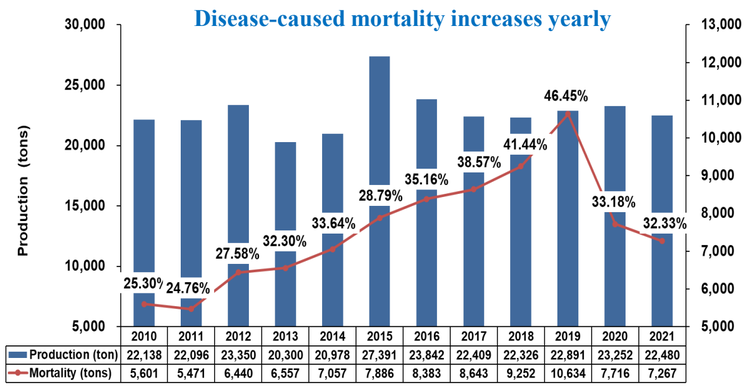
Annual Production Value of different finfish species in Korean Aquaculture



➢ **Diseases affecting Olive flounder aquaculture**

Category	Disease name
Bacteria (30-40%)	*Streptococcosis*
	Tenacibaculosis
	Vibriosis
	Photobacteriosis
	Edwardsiellosis
Virus (30%)	*Viral heamorrhagic septicemia*
Parasites (30-40%)	***Scuticociliatosis***
	Enteromyxosis

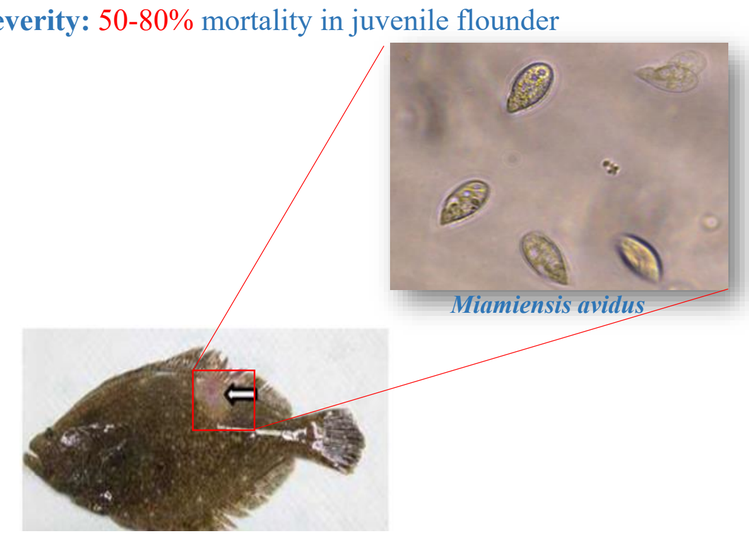
Disease-caused mortality increases yearly



	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Production (ton)	22,138	22,096	23,350	20,300	20,978	27,391	23,842	22,409	22,326	22,891	23,252	22,480
Mortality (tons)	5,601	5,471	6,440	6,557	7,057	7,886	8,383	8,643	9,252	10,634	7,716	7,267

➢ **Scuticocilliatosis disease in Olive flounder aquaculture**

- **Symptom:** Mostly external organs (skin ulceration, hemorrhage, fin rotten, and gill necrosis), sometimes in internal organs including the brain
- **Causative agent:** > 20 species, majorly ***Miamiensis avidus***
- **Severity:** 50-80% mortality in juvenile flounder



Clinical symptom of scuticociliatosis in olive flounder

Materials and Methods

Screening of immersion vaccine adjuvant candidate

Adjuvant candidates:

1. IMS1312
2. IMS1312+PEI
3. IMS1312+Saponin
4. IMS1312+Alum
5. IMS1312+Chitosan
6. IMS1312+β-glucan

Evaluation method:

1. qRT-PCR of mucosal immunity biomarker genes
2. Analysis of skin mucosal protein parameters

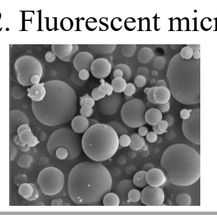
Antigen encapsulation for immersion vaccine delivery

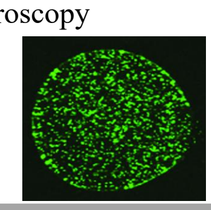
Encapsulation systems:

1. Chitosan MP
2. Chitosan MP +Alginate
3. Alginate MP
4. Alginate MP+Chitosan

Evaluation method:

1. GFP-labeled cell coating
2. Fluorescent microscopy

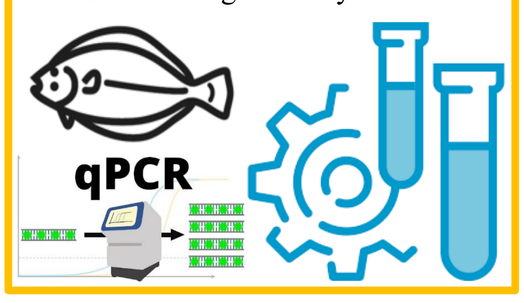




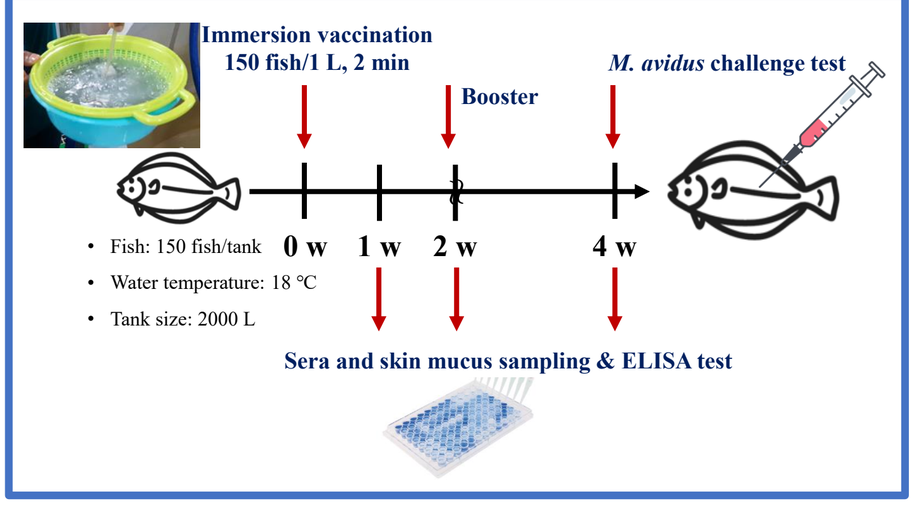
Optimization adjuvant dose & vaccine formulation

Vaccine formulation:

1. Different adjuvant combination
2. Different adjuvant dosage
3. Fish immunization
4. Biomarker gene analysis

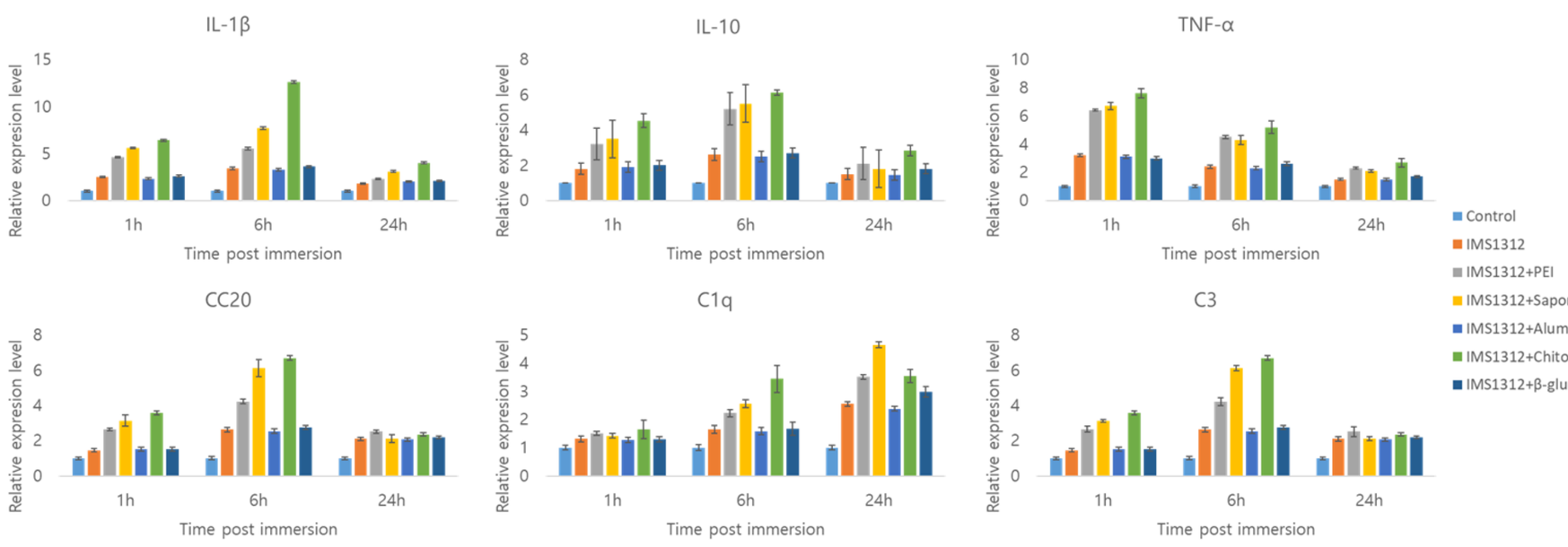


Fish immunization, vaccine efficacy evaluation and ELISA

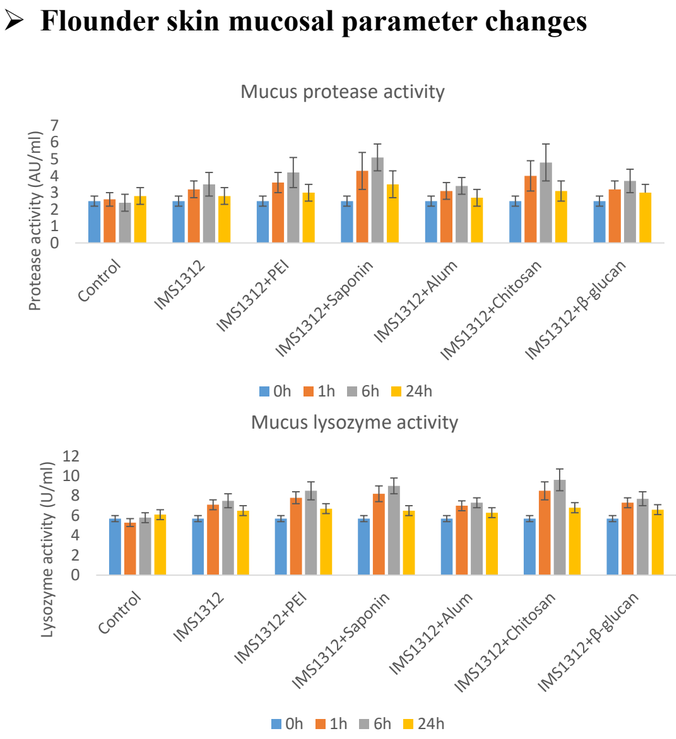


Results

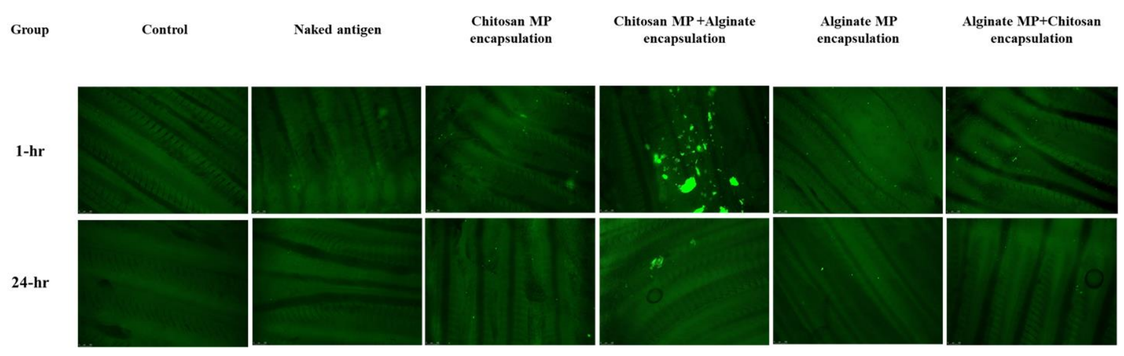
➢ **Mucosal immunity biomarker gene expression profiles in gill tissue**



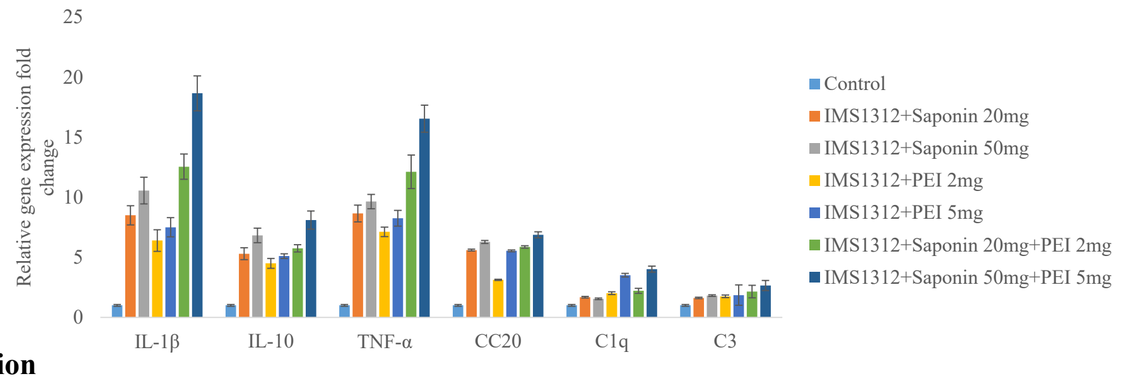
➢ **Flounder skin mucosal parameter changes**



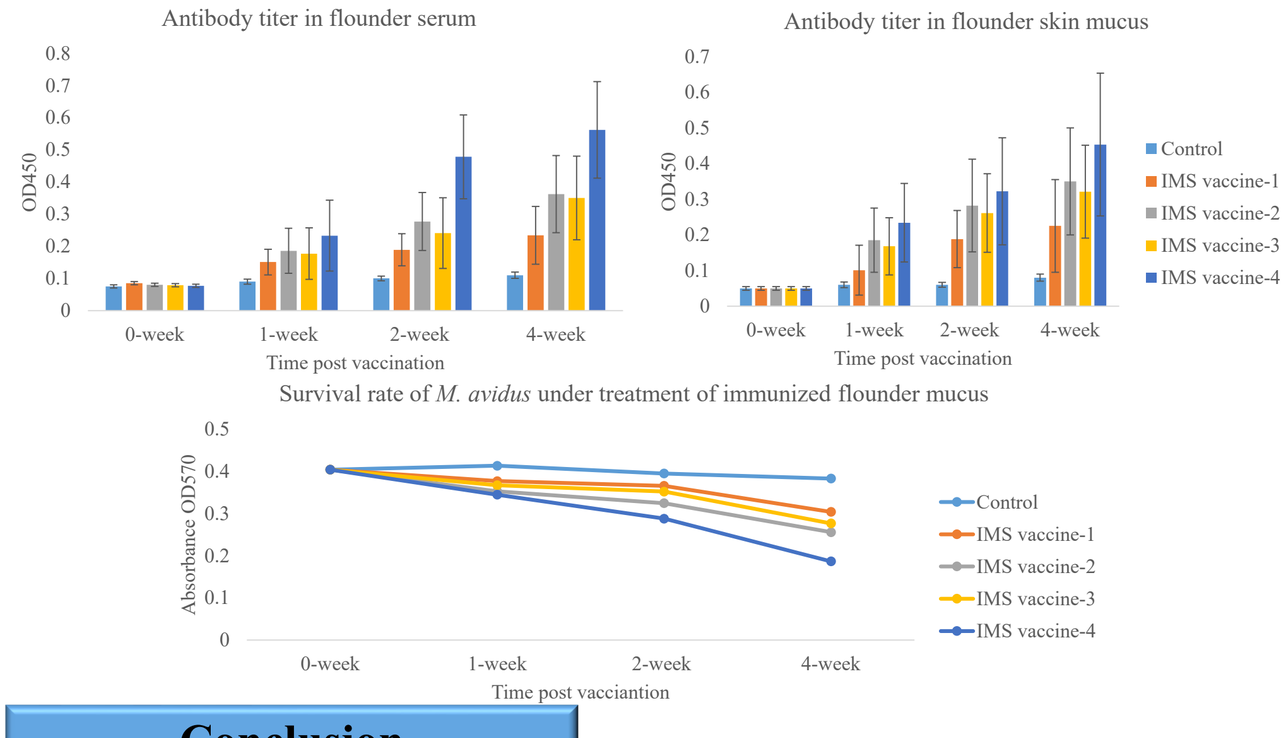
➢ **Effect of different encapsulation methods on antigen intake in immersion vaccination**



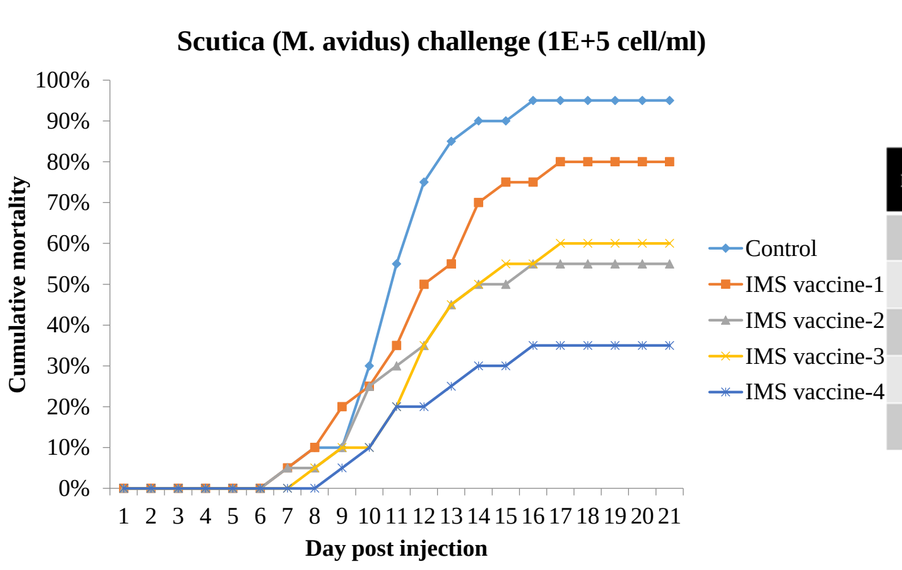
➢ **Biomarker gene responses upon different adjuvant combination and dosages**



➢ **Antibody response and mucosal parasiticidal activity of olive flounder after *M. avidus* immersion vaccination**



➢ **Protective efficacy against *M. avidus* infection of different vaccine formula**



Mortality	RPS60	Final mortality	Final RPS
75%	-	95%	-
50%	33.3%	80%	15.8%
35%	53.3%	55%	42.1%
35%	53.3%	60%	36.8%
20%	73.3%	35%	63.2%

Conclusion

The novel immersion vaccine containing killed *M. avidus* cells encapsulated within chitosan and alginate microspheres, along with a complex adjuvant of PEI and saponin, exhibited the highest mucosal parasiticidal activity, survival rate in challenges, and antibody production. The vaccine developed in this study provides a fundamental strategy for preventing scuticociliatosis and contributes to improving the production of olive flounder aquaculture.