

## Summary of Terminal Evaluation Results

<b>1. Outline of the Project</b>										
<b>Country:</b> The Kingdom of Thailand	<b>Project Title:</b> Development of Aquaculture Technology for Food Security and Food Safety in the Next Generation									
<b>Sector:</b> Agriculture and Rural Development	<b>Cooperation Scheme:</b> Technical Cooperation Project (SATREPS)									
<b>Division in charge:</b> Agricultural and Rural Development Group1, Rural Development Department, JICA	<b>Total Cost</b> (at the time of Evaluation): Approximately: 384 million yen									
<b>Period of Cooperation(R/D):</b> May 25, 2012 to May 24, 2017 (5 years)	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;"><b>Partner Organization:</b></th> <th style="text-align: left;"><b>Country's</b></th> <th style="text-align: left;"><b>Implementation</b></th> </tr> </thead> <tbody> <tr> <td colspan="3" style="vertical-align: top;"> <b>Department of Fisheries (DOF), Ministry of Agriculture and Cooperatives, Kasetsart University (Department of Fisheries, Department of Biochemistry), Chulalongkorn University (Department of Science), Walailak University (School of Agricultural Engineering) Coopeartion Organization : Suranaree University of Technology</b> </td> </tr> <tr> <td colspan="3" style="vertical-align: top;"> <b>Supporting Organization in Japan:</b> Tokyo University of Marine Science and Technology (TUMSAT), Japan International Research Center for Agricultural Sciences(JIRCAS), National Research Institute of Aquaculture                 </td> </tr> </tbody> </table>	<b>Partner Organization:</b>	<b>Country's</b>	<b>Implementation</b>	<b>Department of Fisheries (DOF), Ministry of Agriculture and Cooperatives, Kasetsart University (Department of Fisheries, Department of Biochemistry), Chulalongkorn University (Department of Science), Walailak University (School of Agricultural Engineering) Coopeartion Organization : Suranaree University of Technology</b>			<b>Supporting Organization in Japan:</b> Tokyo University of Marine Science and Technology (TUMSAT), Japan International Research Center for Agricultural Sciences(JIRCAS), National Research Institute of Aquaculture		
<b>Partner Organization:</b>	<b>Country's</b>	<b>Implementation</b>								
<b>Department of Fisheries (DOF), Ministry of Agriculture and Cooperatives, Kasetsart University (Department of Fisheries, Department of Biochemistry), Chulalongkorn University (Department of Science), Walailak University (School of Agricultural Engineering) Coopeartion Organization : Suranaree University of Technology</b>										
<b>Supporting Organization in Japan:</b> Tokyo University of Marine Science and Technology (TUMSAT), Japan International Research Center for Agricultural Sciences(JIRCAS), National Research Institute of Aquaculture										
<b>1-1. Background of the Project</b> <p>In the past three decades, global aquaculture production increased more than 10 times from about 7.2 million tons in 1980 to about 101 million tons in 2014 (Fishery and Aquaculture Global Statistics, FAO). However, as catches from wild capture fisheries leveled off at around 90 million tons a year during the period, increase of the production through aquaculture is inevitable in order to satisfy the growing demand for fishery products due to expansion of the world's population and the change in the dietary habit.</p> <p>To this end, it is quite effective from the standpoint of food security to increase fishery products in the Southeast Asia, one of the world's leading producers of fishery and products, since infrastructures for fishery production has been widely established.</p> <p>Meanwhile, as aquaculture is an economic activity, it is necessary to stimulate the incentives and maintain motivation of fish farmers to ensure its sustainability. Specifically, in Southeast Asia, producing high market-value fish, such as grouper, Asian sea bass, black tiger shrimp, etc., is required to sustain aquaculture through establishing "the aquaculture technology for the next generation" rather than simply expanding current production of low value targets such as tilapia, carp, catfish, vannamei shrimp, etc.</p> <p>However, investment on research and development of "the aquaculture technology for the next generation" creates fiscal burden on the administration of the area, and requires human resources with advanced and high level scientific knowledge. As a result, introduction of such technology has not been achieved as expected yet.</p> <p>Under the circumstances, the Government of Thailand requested the Government of Japan to implement a SATREPS (Science and Technology Research Partnership for Sustainable Development) project for establishing "the aquaculture technology for the next generation" and the request was accepted in 2011.</p> <p>There were some factors behind decision making of the Government of Japan: In recent years, Thai Government has been taking effort to promote the export of fishery products under the slogan of "the kitchen of the world". Thailand is one of the countries in Southeast Asia with capability to perform collaborative research work with Japan to develop the aquaculture technology for the next generation as a result of the past technical transfer that has been conducted by Japan in the field of fishery.</p> <p>After the detailed planning survey in September 2011, and R/D signing by both the Thai and Japanese sides in January 2012, the Project "Development of Aquaculture Technology for Food Security and Food safety in the Next Generation in the Kingdom of Thailand (the Project)" was launched in May 2012 with the cooperation period of five years.</p>										
<b>1-2. Project Overview (PDM (Version 4.0))</b> <p><b>(1) Project Purpose:</b> Advanced technologies for sustainable aquaculture and high quality products are developed in species targeted.</p> <p><b>(2) Outputs:</b></p> <ol style="list-style-type: none"> <li>1. Molecular markers for selective breeding at the molecular level (growth, disease and/or resistance, stress, etc.) are developed.</li> <li>2. Surrogate broodstock technology for aquaculture is developed.</li> </ol>										

3. Practical methods for prevention and control of diseases are developed.	
4. Alternative protein source replacing fish meal and broodstock diets are developed.	
5. Technology for detection and reduction of chemical hazards in aquaculture system is developed.	
<b>(3) Inputs (as of the end of October 2016)</b>	
<b>Japanese Side : Approximately 384 millipn yen</b>	
* Dispatch of Experts:	
<u>Long-term Experts</u> : A total of two Project Coordinators (58.33 M/M) have been assigned.	
<u>Short-term Expert</u> : A total of 18 Short-term Experts (Researchers) (19.2M/M/102trips), in total, have been dispatched.	
<ul style="list-style-type: none"> <li>• Procurement of Equipment : Approximately JPY 159.79 million (THB 54.86 million) (THB1.0=JPY 2.91)</li> <li>• Local Cost Assistance: THB 20.65 million (approximately JPY 60.15 million with the exchange rate THB1.0=JPY2.91)</li> </ul>	
<b>Thai Side</b>	
* Allocation of C/Ps: A total of 116 C/Ps. 93 C/Ps as of the end of November 2016	
* Office space in DOF in Bangkok, and facilities (laboratories and facilities for fish rearing) of DOF and participating universities	
* Operation Cost: THB 50.83 million (approximately JPY 147.92 million with the exchange rate THB1.0=JPY2.91) on the Project activities	
<b>2. Terminal Evaluation Team</b>	
<b>Japanese Side</b>	<b>Cameroonian Side</b>
<p>(1) Ms. Satoshi CHIKAMI (Leader), Senior Advisor, Rural Development Department, JICA</p> <p>(2) Mr. Atsushi KANO (Cooperation Planning), Team2, Agricultural and Rural Development Group1, Rural Development Department, JICA</p> <p>(3) Dr. Hideaki HIGASHINO (Evaluation Analysis), Senior Consultant, RECS International. Inc.</p> <p>(4) Dr. Makie KOKUBUN (observer) ST Research Supervisor/ Professor Emeritus, Tohoku University</p> <p>(5) Ms. Mizuki KAWASAKI (observer) Department of International Affairs, JST</p>	<p>(1) Dr. Nuanmanee Pongthana (Leader), Senior Expert, DOF</p> <p>(2) Dr. Uthairat Na-Nakorn, Professor, Kasetsart University</p> <p>(3) Mr. Banchong Amornchewin, Director, Planning and Management Branch, TICA</p>
<b>Period of Review:</b> From 14 to 28, November 2016	<b>Type of Evaluation:</b> Terminal Evaluation
<b>3. Results of Evaluation</b>	
<b>3-1. Project Performances</b>	
<b>Summary of Project Purpose Achievements</b>	
<i>Project Purpose: Advanced technologies for sustainable aquaculture and high quality products are developed in species targeted.</i>	
<i>Indicator 1: The number (at least 3) of species targeted on improved aquaculture technologies</i>	
<ul style="list-style-type: none"> <li>• Indicator 1 has been achieved. Various improved aquaculture technologies have been developed or are under development for nine target species at the time of Terminal Evaluation.</li> </ul>	
<i>Indicator 2: The number (at least 60% of members) of researchers who acquired skills of advanced aquaculture technologies</i>	
<ul style="list-style-type: none"> <li>• Indicator 2 has been achieved. During the cooperation period, a total of 58 Thai researchers, about 60% of the total Thai researchers currently involved in the Project (93 researchers), were dispatched to Japan for training. After participating in the training in Japan, they have conducted research works utilizing knowledge and experiences obtained in the training and extended their knowledge to fellow researchers.</li> </ul>	
<i>Indicator 3: The number (at least 50) of scientific journal, technical reports, educational brochures, conference proceedings/abstracts, and/or newsletters.</i>	
<ul style="list-style-type: none"> <li>• Indicator 3 has been satisfied at the time of Terminal Evaluation as the number of scientific papers prepared by the Thai and Japanese researchers of the Project has reached 75. Thai researchers prepared 35 out of 75 scientific papers as lead authors.</li> </ul>	
<i>Indicator 4: The number (at least 10) of workshops and/or seminars for education of skills and/or dissemination of a project outputs</i>	
<ul style="list-style-type: none"> <li>• Indicator 4 has been achieved. The number of seminars, meetings, and workshops held were 26.</li> </ul>	
<b>Summary of Output Achievements</b>	
<b>Output1: Molecular markers for selective breeding at the molecular level (growth, disease resistance, stress) are developed.</b>	
<b>Indicator</b>	<b>Achievements</b>

<p><i>Indicator1-1: DNA markers (at least 2) related to economically important traits are developed.</i></p>	<ul style="list-style-type: none"> <li>* Indicator 1-1 has been achieved.</li> <li>* Selection of useful DNA markers were conducted for tiger grouper, giant grouper, shrimp (black tiger and vannamei), and Asian seabass.</li> <li>* Grouper: 11DNA (tiger grouper) and 2 DNA (giant grouper) for fast-growth trait were developed.</li> <li>* For black tiger shrimp: Useful markers for disease (WSSV) resistance (one SNP marker) and fast-growth traits (two SNPs markers) were identified for the established families under the Project.</li> </ul>
<p><i>Indicator1-2: At least five informative DNA markers for parental analysis are developed.</i></p>	<ul style="list-style-type: none"> <li>* Indicator 1-2 has been achieved</li> <li>* Grouper: Nine loci of informative DNA markers (microsatellites) were obtained for parental analysis.</li> <li>* Shrimp: Seven loci of informative DNA markers (SNPs and microsatellites) were selected for vannamei shrimp for parental analysis.</li> <li>* For black tiger shrimp, five SNPs markers were selected for parental analysis.</li> <li>* Asian Sea Bass: Total 560 DNA samples of 80 brood fish and 84 progenies of Asian seabass had been applied with 35 informative microsatellite markers (35 loci) until May 2016.</li> <li>* All genotyping data (35 loci) of Asian seabass are on the process of parental analysis.</li> </ul>
<p><i>Indicator2: Evaluation technique for detecting useful traits is established.</i></p>	<ul style="list-style-type: none"> <li>* Indicator 2 has been almost achieved.</li> <li>* As for grouper, evaluation of traits related to growth has been under progress, and findings have been obtained through length and weight measurement on monthly basis.</li> <li>* For shrimp, artificial infection methods for evaluation of DNA markers were developed against WSSV and EMS.</li> <li>* Asian Sea Bass: Progeny testing technique were attempted to be used to perform parental analysis to identify the putative parents that give hypoxia tolerance, bacterial disease resistance and good growth progeny.</li> <li>* However, correction is being made on genotypic data, and the technique has not been established yet.</li> </ul>
<p><i>Indicator3: At least one genetic linkage map is established.</i></p>	<ul style="list-style-type: none"> <li>* Indicator 3 has been achieved.</li> <li>* The genetic linkage maps of tiger grouper with 183 DNA markers and giant grouper with 130 DNA markers were developed from hybrid grouper in 2015.</li> </ul>
<p><i>Indicator4: At least two families from target species with economically important traits are identified.</i></p>	<ul style="list-style-type: none"> <li>* Indicator 4 has been achieved.</li> <li>* Three tiger grouper families and 1 hybrid (male giant grouper x female tiger grouper) family were produced (2012). Measuring monthly growth data of above families throughout the year after insert microchip ID was obtained (2013).</li> <li>* An F2 family of tiger grouper was selected in October 2015, and fattened to the broodstock for producing the next generation (researchers are waiting for the mating/spawning season from the end of 2016 to early 2017).</li> <li>* Shrimps: Fast-growth and WSSV resistant families were established in black tiger shrimp.</li> <li>* Asian Seabass: Families of Asian seabass have not been established yet. Parental analysis using all genotyping data so far obtained is under progress.</li> </ul>

**Output2: Surrogate broodstock technology for aquaculture is developed.**

<b>Indicator</b>	<b>Achievements</b>
<p><i>Indicator 1: Cell transplantation method is individually developed for target species.</i></p>	<ul style="list-style-type: none"> <li>* Indicator 1 has been achieved.</li> <li>* Targeting striped catfish (recipient) for Mekong giant catfish and tiger grouper (recipient) for giant grouper, cell transplantation method and the related techniques (fluorescent labeling technique of oogonial cells, microinjection technique,</li> </ul>

	<p>determination of colonization of the fluorescent donor cells in the gonad of recipient fish, and characterization of the morphology of PGC (Primordial Germ Cell) and donor transplanted oogonial cells in recipient gonad) were developed and transferred.</p> <ul style="list-style-type: none"> <li>* As of November 2016, 139 micro-injected larvae are under rearing (two months after micro-injection), and future examination of effectiveness of the surrogate broodstock technologies will be conducted using DNA markers.</li> <li>* Micro-injected larvae of striped catfish are also under rearing for further examination.</li> </ul>
<i>Indicator 2:</i> Recipient species are identified for their host species.	<ul style="list-style-type: none"> <li>* Indicator 2 has been almost achieved.</li> <li>* Candidate recipient species were chosen: striped catfish (<i>Pangasianodon hypophthalmus</i>) for Mekong giant catfish and tiger grouper (<i>Mycteroperca tigris</i>) for giant grouper.</li> <li>* Appropriate timing of transplantation was clarified through examination of timing of PGC (Primordial Germ Cell) migration.</li> </ul>
<i>Indicator 3:</i> Compatibility between donor and recipient is confirmed.	<ul style="list-style-type: none"> <li>* Indicator 3 has been almost achieved.</li> <li>* Compatibility between targeted donors (Mekong giant catfish) and recipients is under observation.</li> <li>* After transplantation of the donor cells into the undifferentiated reproductive gland of tiger grouper, donor-originated germ cells were traced to evaluate the donor-recipient relationships.</li> <li>* The full length of vasa cDNA of tiger grouper was characterized, and its expression in various tissues was conducted.</li> </ul>

**Output3: Practical method for prevention and control of diseases are developed.**

<b>Indicator</b>	<b>Achievements</b>
<i>Indicator 1:</i> At least 2 profiling array for gene expression are developed.	<ul style="list-style-type: none"> <li>* Indicator 1 has been achieved.</li> <li>* Sampling of six species (giant grouper, tiger grouper, Asian seabass, black tiger shrimp, vannamei shrimp, and Nile tilapia) was conducted in Thailand.</li> <li>* Using the samples, gene catalogue for the six species was developed at TUMSAT, Japan.</li> <li>* Two profiling of differentially expressed genes of Taura syndrome virus and WSSV resistance shrimp were obtained as planned. These include several candidate genes for selection of disease resistance shrimp.</li> <li>* In addition, a profile of genes that might be involved in response to the YHV infection has been obtained by suppression subtractive hybridization.</li> </ul>
<i>Indicator 2:</i> At least 10 candidate immune genes of fish and shrimp are identified and characterized.	<ul style="list-style-type: none"> <li>* Indicator 2 has been achieved.</li> <li>* Genes and proteins associated with <i>V. parahaemolyticus</i> AHPND were identified and characterized from black tiger shrimp.</li> <li>* Site-directed mutagenesis at the conserved nucleotide of selected TF-binding sites revealed the putative binding site for activator on ALFPm6 promoters for understanding the resistance of WSSV infection. The putative transcription factor binding site of ALFPm6 gene was identified.</li> <li>* A total of 11 candidate immune genes were identified and characterized for black tiger shrimp:</li> <li>* Significant numbers of immune related genes of giant grouper, tiger grouper, and Asian sea bass were identified at TUMSAT.</li> </ul>

<p><i>Indicator 3:</i> At least 2 candidate antigens of vaccines for pathogenic microorganisms are identified.</p>	<ul style="list-style-type: none"> <li>* Indicator 3 has been achieved.</li> <li>* The Project researchers identified 10 candidate antigens from <i>Streptococcus agalactiae</i> for subunit vaccine development for tilapia.</li> <li>* Three antigenic proteins (pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase and cell wall surface anchor family protein) were chosen to develop subunit vaccine.</li> <li>* Group B streptococcal surface immunogenic protein (sip protein) was also chosen, which was previously reported as a good subunit vaccine, for vaccine development.</li> </ul>
<p><i>Indicator 4:</i> At least one practical vaccine for pathogenic microorganisms is developed.</p>	<ul style="list-style-type: none"> <li>* Indicator 4 has been achieved.</li> <li>* Marine shrimp vaccine for <i>V. parahaemolyticus</i> and WSSV was developed and tested.</li> <li>* Using the <i>V. parahaemolyticus</i>'s formalin-killed vaccine, the survival of shrimp challenged by <i>V. parahaemolyticus</i> was statistically different from the control that 0% survived.</li> <li>* In the similar approach, WSSV vaccine was also developed and the primary test showed that the vaccine could prevent WSSV infection.</li> <li>* As for tilapia, from DNA vaccine efficacy determination, it revealed that those subunit vaccines did not demonstrate good protective effects against streptococcosis disease. This resulted from extremely low translation rate from DNA vaccine in target animals which affect the antigenic protein production and immune stimulation.</li> <li>* DNA and formalin killed bacterial cells vaccine trials in the field (Kamphaeng Phet, Kanchanaburi, and Nong Khai) were executed in March and November 2015, to verify the effectiveness.</li> <li>* Vaccine of combined serotype Ia and III has been established to be used as functional vaccine for tilapia culture in Thailand.</li> <li>* Results from field trial showed very promising result but more trials are needed with higher efficacy vaccine. The Project researchers are now developing a novel DNA vaccine carrier to improve vaccine efficiency.</li> <li>* Feasibility of commercial production of vaccine including licensing procedure is ongoing.</li> </ul>
<p><i>Indicator 5:</i> Practical methods for health management of fish and shrimp are developed.</p>	<ul style="list-style-type: none"> <li>* Indicator 5 has been almost achieved.</li> <li>* Genome analyses were conducted in terms of bacteria causing EMS and AHPND in Southeast Asian countries including Thailand.</li> <li>* Based on the analyses, PCR test procedures (diagnostic procedures) were developed and its effectiveness was verified.</li> <li>* The diagnostic procedures were regarded as standard methods of DOF in 2014 and was adopted by OIE as a standard method in September 2016.</li> <li>* DOF made the achievement public via a media release in June 2014.</li> <li>* It was verified that rALFPm3-supplement diet increases survival rate of <i>Vibrioparahaemolyticus</i> AHPND-infected shrimp, and the method for large scale production of ALFPm3 is currently developed.</li> <li>* The ALFPm3-supplement diet for shrimp was prepared by mixing crude rALFPm3 with shrimp mash feed.</li> </ul>

**Output4: Alternative protein source replacing fish meal and broodstock diets are developed.**

Indicator	Achievements
<p><i>Indicator 1:</i> Broodstock diets for maturation of shrimp is developed.</p>	<ul style="list-style-type: none"> <li>* Indicator 1 has been achieved.</li> <li>* The study on variation in biochemical composition, fatty acids, amino acids profile and vitamin E in eggs and tissues of wild</li> </ul>

	<p>banana shrimp (<i>Penaeus merguensis</i>) in different stages of sexual maturation was completed. Based on this information, the diets for the feeding trials were formulated.</p> <ul style="list-style-type: none"> <li>* The feeding trial was conducted to investigate the supplemental effects of vitamin E, astaxanthin, taurine on reproductive performance of banana shrimp broodstock. The obtained results will be applied to the next feeding trial, which will be held in the 3<sup>rd</sup> year of the project, in order to evaluate effectiveness of combination of taurine, astaxanthin and vitamin E as ingredients of banana shrimp broodstock diet.</li> <li>* Based on the results of experiments of feeding trials, broodstock diet containing 19.8 % of fish meal, reduced from 28%, was produced in Japan. Along with it, it was confirmed that the change of fish meal content did not have significant effect on the amount of intake.</li> <li>* As a result, alternative protein sources (squid meal (SQM), corn gluten meal (CGM), and de-hulled soybean meal (DSBM)) were identified, out of 9 candidate protein sources tested (SQM, krill meal, shrimp head meal, lupine meal, DSBM, soybean meal, poultry by-product meal (PBM), soybean protein concentrate (SPC), and CGM).</li> <li>* Feeding trials were repeated three times until October 2016, and a prototype of broodstock diet was produced.</li> <li>* For mass production, negotiation with private manufacturers is ongoing.</li> </ul>
<p><i>Indicator 2:</i> Alternative diets for shrimp and fish are developed.</p>	<ul style="list-style-type: none"> <li>* Indicator 2 has been almost achieved.</li> <li>* For vannamei shrimp, optimum level of selected plant protein feed ingredients was determined by using IGF-I expression.</li> <li>* The IGF-I cDNA expression in vannamei shrimp fed with optimum level of selected plant protein feed ingredients were obtained in August 2015.</li> <li>* Real-time PCR analysis has been completed in November 2016 and data is under analyses.</li> <li>* For tiger grouper, alternative protein sources digestibility efficiency of tiger grouper was obtained in January, 2015.</li> <li>* A combination of 18% SPC, 25% CGM &amp; 30% PBM as a replacement for about 100% of FM diet for tiger grouper produced growth comparable to the fish meal diet at about 50% dietary protein level.</li> <li>* Evaluation of prototype fish meal replacement diet of tiger grouper (<i>Epinephelus fuscoguttatus</i>) in commercial culture scale was conducted in January 2016 and</li> <li>* FCR (feed conversion rate) of tiger grouper fed non-fish meal diet was better than that on trash fish. And survival rates between two feed types were similar (84.50-85.50%).</li> <li>* The non-fish meal feed replaced with SPC, CGM and PBM in proper combination from the results of the second experiment was promoted to private tiger grouper cage farm in Phuket Province, Thailand.</li> </ul>

**Output5: Technology for detection and reduction of chemical hazards in aquaculture system is developed.**

Indicator	Achievements
<p><i>Indicator 1:</i> At least one prototype of identification kit for detection of hazard chemicals is developed.</p>	<ul style="list-style-type: none"> <li>* Indicator1 has been achieved.</li> <li>* Optimum protocol for detecting Leucomalachite Green (LMG) by ELISA method was developed in April 2014. The protocol was designed to have the sufficient sensitivity (less than 2ppb), recovery rate (75-120%), and repeat accuracy (CV will be less than 10%).</li> <li>* Changes of gene expression in fish exposed to LMG were examined and suitable genes were identified for the monitoring in June 2014.</li> </ul>

	<ul style="list-style-type: none"> <li>* Based on the information above mentioned, a prototype tool kits for detecting LMG was developed.</li> <li>* Criteria of ELISA kit was set for LMG residue in farmed fish and new revision of monitoring program for LMG residues in farmed fish is ready to apply.</li> <li>* Colorimetric detection for chemical residual is established. The validation of detection system in aquaculture production is being established after the detection module is optimized.</li> <li>* One nucleotides specifically recognized nitrofurantoin which can be further used and develop simple detection system was obtained.</li> </ul>
<p><i>Indicator 2:</i> Technologies for reduction of chemical contamination in aquaculture system are developed.</p>	<ul style="list-style-type: none"> <li>* Indicator2 has been almost achieved.</li> </ul> <p><u>Biological Approach</u></p> <ul style="list-style-type: none"> <li>* Malachite-decomposing (decolorizing) bacteria were isolated from the water, sediment and fish samples as well as fermented food products.</li> <li>* Strain J29-3 showed the highest MG decolorizing efficiency (80%) in nutrient broth containing 500 ppm MG after incubated at 37 degrees for 48 hours.</li> <li>* Strain J29-3 was characterized and identified to be <i>Pandoraea pnomenusa</i></li> <li>* In the 2<sup>nd</sup> half of the Project, further analysis was conducted in terms of utilization of those strains.</li> <li>* Optimum conditions for growth and MG decolorization as well as other beneficial information of <i>P. pnomenusa</i> J29-3 in culture media were obtained.</li> <li>* J29-3 was applied into the simulated fish pond environment model which be artificially contaminated with malachite green. The total bacteria count, total malachite green decolorizing bacteria, malachite green and leucomalachite green concentration in water sample were determined at selected time intervals.</li> <li>* The preliminary result indicated that <i>P. pnomenusa</i> J29-3 has a benefit potential for the application on malachite green reduction in aquaculture system.</li> <li>* During the remaining cooperation period, the best practical approach for application of J29-3 in fishponds will be determined.</li> </ul> <p><u>Chemical Approach</u></p> <ul style="list-style-type: none"> <li>* A comparison study of adsorption percentage of various adsorbents for 100 ppm LMG. Activated charcoal showed the highest adsorption percentage, and zeolite was the second effective adsorbent for LMG adsorption. Zeolite was selected to remove LMG because its adsorption ability and cost.</li> <li>* Addition of 1% zeolite (w/w) in the 100 ppm MG solution, MG was removed around 98%.</li> <li>* Addition of 1% zeolite (w/w) in the 100 ppm LMG solution, MG was removed around 99%.</li> <li>* The optimum concentration of Zeolite was applied for reduction of LMG in Tilapia muscle and culture water. From the preliminary result, Tilapia fries were cultured in 1 ppb MG solution for 72 hrs. and LMG was also detected in the fry at 0.255 ppb.</li> </ul>

### 3-2. Summary of Evaluation based on Five Evaluation Criteria

Evaluation results based on 5 evaluation criteria are as follows:

#### Relevance: High

The Project was evaluated as highly relevant with Thai development policy and Japan's aid policy and strategy, at the time of Terminal Evaluation.

#### Effectiveness: High

Effectiveness of the Project is evaluated high as the Project activities have been implemented as scheduled and some remarkable achievement were reported. In addition, indicators of Project Purpose were reasonably achieved.

Meanwhile, implementation of all the five research topics was limited to tiger grouper, and some of the research activities are still at the laboratory stage, and it will take a certain period of time until their practical application in the field will be made.

Taking the situation into consideration, the Project researchers are expected to make a further effort to integrate developed technologies and research outcomes into “advanced technologies for sustainable aquaculture and high quality products” during the remaining cooperation period and beyond, making most of the technologies they have acquired in the Project.

**Efficiency: Relatively High**

Both the Thai and Japanese sides made input reasonably which resulted in satisfactorily output generation. In particular, intensive training in Japan in the initial stage of the Project was effective for smooth start of research activities thereafter in Thailand. The equipment provided by the Japanese side has been fully utilized by the Thai C/Ps for the research activities and maintained properly.

Nevertheless, the complicated institutional set-up of the Project with more than 100 Thai researchers under 13 research groups, and frequent change of the Project Director, time consuming document approval procedure in DOF, made difficult the management of the Project, and slightly lowered the efficiency.

**Impact: Significant Technical Impact**

Impact of the Project, especially in the technical impact on aquaculture in Thailand, is considered significant.

For example, the Project developed the PCR test procedures (diagnostic procedures) for EMS/AHPND which were adopted as a standard diagnostic procedure of DOF (2014) and OIE (2016). They are used all over Thailand.

**Sustainability: Relatively High**

Sustainability of the Project is considered satisfactory, except for slight concern in institutional aspect at the time of the Terminal Evaluation.

As for the management of the Project (Project Director and Manager) there has been frequent turnover. In terms of researchers, there was turnover, not significantly, at local research institutions. After the Project terminates, it is apprehended that more turnover will occur.

Research activities to establish “the aquaculture technology for food security and food safety in the next generation in Thailand” is a challenging mission for researchers of DOF and relevant research institutions. It is expected that full-scale support by the Government of Thailand will be provided from long-term point of view, to enable the researchers to continue their research activities until they achieve their targets.

**3-3. Factors promoting the production of effects**

**3-3-1. Factors pertaining to planning**

None

**3-3-2. Factors pertaining to implementation process**

**(1) Intensive training in Japan and follow-up by the Japanese researchers**

At the initial stage of research activities, training of experienced researchers, who were supposed to take leadership in each research group, was conducted in Japan, then, trainings of young researchers followed. Additionally, follow-up instruction for ex-trainees were conducted when Japanese researchers visited Thailand. This combination contributed to not only transfer of advanced technologies to Thai researchers but also smooth implementation of the research activities in the Project.

**(2) Collaborative Relationship among the Japanese and Thai Research Organizations**

Long time collaborative relationship among TUMSAT and Thai research institutions such as Kasetsart and Chulalongkorn universities contributed to smooth execution of the Project over the cooperation period.

**(3) Commitment of Thai C/Ps**

Thai side assigned capable and hardworking C/Ps to the Project. Commitment of Thai C/Ps contributed to achieving research activities.

**(4) A Japanese Researcher stationed in CFRDC, Krabi**

A researcher, who graduated from Tokyo University of Marine Science and Technology, has been stationed in DOF Krabi since December 2012, and contributing to technical instruction to Thai C/Ps and information sharing with the Japanese side.

**3-4. Factors inhibiting the production of effects**

**3-4-1. Factors pertaining to the implementation process**

The Project has been moving forward on schedule as a whole, the Team recognizes no significant factors that inhibited the progress of the Project at the moment of the Terminal Evaluation.

However, it is pointed out that the facts described below slightly lowered the Project efficiency.

- Complicated institutional set-up with more than 100 Thai researchers under 13 research groups encompassing DOF (HQs in Bangkok and 11 local research institutions), Kasetsart University (Departments of Fisheries and Science), Chulalongkorn University (Department of Science), Walailak University (Center for Excellence for Shrimp), and Suranaree University of Technology (School of



Production Technology)

- Insufficient knowledge of the researchers in terms of project management using PDM and PO
- Change of the Project Director (three times in four and a half years)
- Time consuming process of document approval within DOF

### **3-5. Conclusion**

Evaluation of the Project based on five evaluation criteria was satisfactory. The Evaluation Team concludes that the Project has made good progress so far and will have reasonably achieved the Project Purpose by the end of the Project cooperation period; May 2017. Hence, it is concluded that the Project will be terminated in May 2017 as described in R/D signed in January 2012.

### **3-6. Recommendations**

#### **3-6-1: Recommendations to the Project Team**

(1) Envisioning “the aquaculture technology for food security and food safety in the next generation” among the Project Researchers

For efficient and effective planning on the future research and development in the field of aquaculture in Thailand, it is preferred that the Thai researchers will clearly envision and share the common concept of the “aquaculture technology for food security and food safety in the next generation”, with reference to the 12<sup>th</sup> National Economic and Social Development Plan(2017-2021), “Life below Water” in Sustainable Development Goal of UN (2015), and so on.

(2) Confirmation of the research activity progress and the prospect of their practical utilization

During the remaining cooperation period, the Project Team is recommended to confirm the progress of the research activities under the five Output in terms of each target species, and to prepare concrete strategy on how to put the developed technologies into practical utilization.

(3) Intellectual property rights

As for the developed methodologies and materials that are possible to put into practical utilization within a short-term, the Project Team is recommended to discuss on the acquisition of the intellectual property rights.

#### **3-6-2: Recommendations to the Thai Side**

(1) Utilization of the research network developed through the implementation of the Project

Through the Project implementation, a valuable research network was established among relevant researchers, not only among Thai researchers, but also between Thai and Japanese researchers. The Thai side is recommended to use the network effectively to handle with the research topics in the future.

(2) Promotion and dissemination of research outcomes

In order to disseminate the research outcomes of the Project to the researchers and the shrimp and fish farmers in Thailand, the Thai side (with DOF as the core organization), is recommended to strategically execute promotion and dissemination of the research outcomes.

(3) Technical assistance to the neighboring countries

The Thai side is recommended to conduct technical assistance to the neighboring countries, where similar aquaculture is conducted, to disseminate the advanced technologies developed under the Project, thereby contributing to the increase of aquaculture production in Southeastern Asia.

#### **3-6-3: Recommendations to the Japanese Side**

(1) Utilization of the research network developed through the implementation of the Project

The Japanese side, with TUMSAT as the core institution, is recommended to give technical guidance and assistance to the Thai side as necessity rises to ensure the sustainability of the Project, through the collaborative research network formulated through the Project implementation as mentioned (3-6-2 (1))

### **3-7. Lessons Learned**

(1) Intensive Training in the early stage of the Project

The trainings, conducted intensively in the early stage (the 1<sup>st</sup> and 2<sup>nd</sup> years) of the Project, contributed to smooth and efficient operation of the research activities in Thailand during the entire cooperation period.

(2) Training of young researchers

Young researchers, accounting for almost two thirds of all the Thai C/Ps, improved their knowledge and skills through participation in the trainings in Japan and technical guidance from the Japanese researchers, and are expected to contribute to the further research activities in the field of aquaculture in Thailand in the future.

(3) Document approval procedure of the recipient countries

In the Project, it was not until November 2016 that MTA (Material Transfer Agreement) had been signed due to repeated organizational re-structuring of DOF, and complicated and time consuming legal procedures, although MTA is prerequisite for a project to deal with biological material transfer.

It is necessary for a project that will handle with biological material transfer, to proceed necessary legal procedures including MTA signing as early as possible.