



# Microalgae Research in Thailand and Southeast Asia



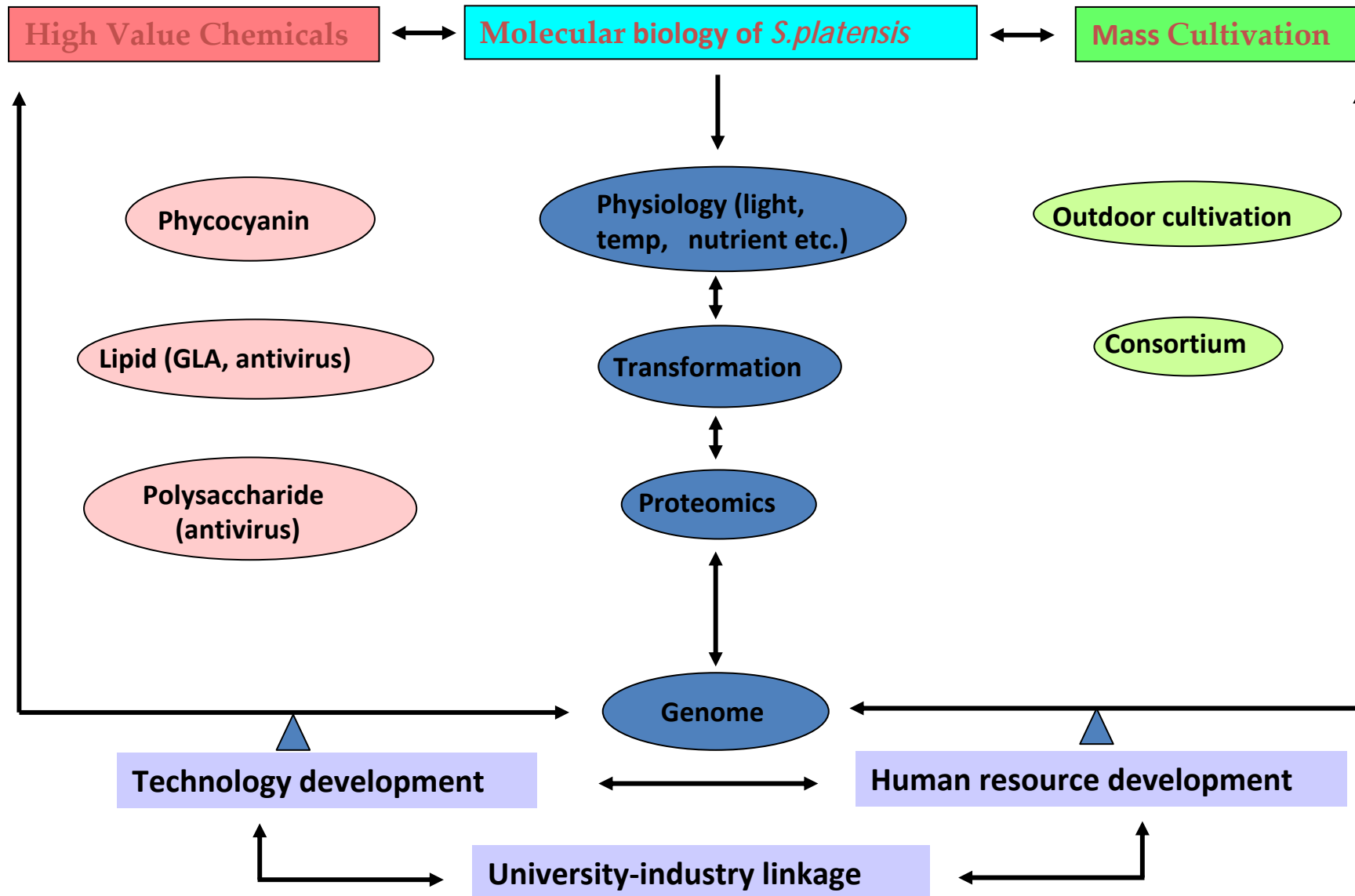
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**November 2<sup>nd</sup>, 2009, Berlin**

# Outline

- Researches at King Mongkut's University of Technology Thonburi (KMUTT), Thailand
- Commercial plants in Thailand
- Researches in Thailand
- Researches in other SE countries

# KMUTT Framework of Algal Biotechnology Group



# Mass Cultivation



*Chaetoceros*



*Chlorella sp.*

## Spirulina, Green Algae & Diatom



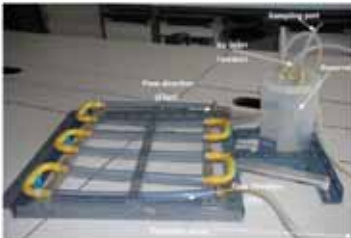
Cultivation management

Strain selection & improvement

Reactor design &  
Engineering systems

Optimization

- medium composition
- cell density
- mode of cultivation (batch & semi-cont.)



### Goal:

- High yield & low cost of production
- *Spirulina* with high
  - protein
  - GLA
  - phycocyanin
- Green algae & diatom with high lipid

# Cultivation ponds and photobioreactors



0.5 m x 1.5 m x 0.3 m (0.15 m), Working volume  
~ 100 L



0.6 m x 3.0 m x 0.3 m (0.15 m), Working volume ~  
200 L



The outdoor tubular  
photobioreactor of 1.4 m<sup>2</sup> surface  
area, 4 cm diameter and 150 L  
working volume



Glass tubular photobioreactor of  
0.229 m<sup>2</sup> surface area, 3 cm  
diameter and 120 L working  
volume.

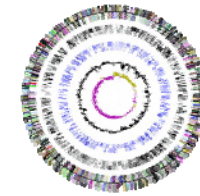
# Molecular biology of *Spirulina*

- Gene expression and regulation of GLA production
- Transformation system
  - development of a gene transfer system in cyanobacteria & *Spirulina*
  - factors influence to efficiency and stability of transformation
  - strategies to get high efficiency and stable transformation system of *Spirulina*
- Proteomics
  - Database and tools for protein identification using PMF
  - Proteome analysis of *Spirulina* in response to low temperature stress
  - *Spirulina* proteome : high temperature response; study of regulatory regions and regulatory proteins of desaturase genes
- *Spirulina* genome
- Microarray
  - study of genome-wide gene expression  
(to understand how cells respond to environmental stress)
  - assignment of functions to new genes  
(based on similarities in expression patterns with those of known genes )
- Metabolic flux analysis

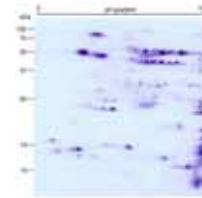




# Algal Biotechnology Research Unit @ KMUTT



Genome project



Proteome



*In silico* Modeling/metabolic pw



Metabolome/lipidome



*Spirulina* Consortium/consultancy



Transformation Development



Strain selection/Improvement



High value compound extraction



GLA



Mass cultivation technology

Year 1980

> 20 years

2009



# Researches in Thailand

- **Culture collection of microalgae in Thailand**  
Taxonomy
- **Biotechnology** (*Spirulina* genome, mass cultivation for health food, animal feed, bioactive compounds (polysaccharide, PUFA-HUFA), extraction process: oil, pigments – phycocyanin, astaxanthins, carotenoid,  $\beta$ -carotene)
- **Wastewater treatment** (*Chlorella*, *Spirulina* from tuna cane, palm oil, pig farm, rubber processing etc.)
- **Aquaculture** (*Spirulina*, diatom (*Skeletonema*, *Chaetoceros*, *Thalassiosira* etc.)
- **Environment** (Biodiversity , toxicity index)

# Microalgal Oil

- Recently funded by the Petroleum Authority of Thailand (PTT) to various universities and research institutes in Thailand
  - Strain selections
  - Screening methods
  - Physiology of selected strains
  - Cultivation techniques
  - Harvesting, extraction processes

# **Microalgal Culture collection in Thailand**

Freshwater strains : Thailand Institute of Science and Technology Research (TISTR)- green algae and cyanobacteria

Marine strains : Bangsean Institute of Marine Science (BIMS)- green algae and diatoms

Others : Collection of university laboratories

Species	Lipid (% dry matter)	
	Literatures	Thailand
<i>Botryococcus braunii</i>	25-75	
<i>Chaetoceros gracilis</i>		50-60 (KMUTT&PTT)
<i>Chlorella</i> sp.	28-32	
<i>Chlorella emersonii</i>	63	
<i>Chlorella minutissima</i>	57	
<i>Chlorella protothecoides</i>	23/55	
<i>Chlorella sorokiana</i>	22	
<i>Chlorella vulgaris</i>	14-40/56	50-60 (KMUTT&PTT)
<i>Cryptothecotridium cohnii</i>	20	
<i>Dunaliella salina</i>	14-20	
<i>Dunaliella binocolata</i>	8	
<i>Isochrysis</i> sp.	25-33	45 (KMUTT&PTT)
<i>Neochloris oleoabundans</i>	35-65	
<i>Nitzchia</i> sp.	45-47	30 (KMUTT&PTT)
<i>Scenedesmus acutus</i>		50 (MU&PTT, 2009)
<i>Scenedesmus dimorphus</i>	6-7/16-40	
<i>Scenedesmus obliquus</i>	11-22/35-55	20 (KMUTT&PTT)
<i>Spirulina maxima</i>	4-9	
<i>Spirulina platensis</i>		10-12 (KMUTT&PTT)
<i>Tetraselmis sueica</i>	15-20	14 (KMUTT&PTT)

## **Wastewater Utilization:**

Utilization of Tapioca Wastewater-Funded by the National Center for Biotechnology and Genetic Engineering (1986)

Wastewater from pig farm  
(KMUTT-Funded by the World Bank,  
2007-2008 under the project  
'Management of Pig Farm Wastes')

Wastewater from palm oil industry, tuna  
canning industry

# Cultivation ponds using tapioca starch wastewater



# Cell Harvesting



# Pig manure effluent from Large Pig Farm (from UASB) (Rajburi, Thailand)



# Treatment of waste from small pig farm



# Demonstration of cultivation at the small pig farm



# Demonstration of cultivation at the small pig farm



# Demonstration of cultivation process at the large pig farm



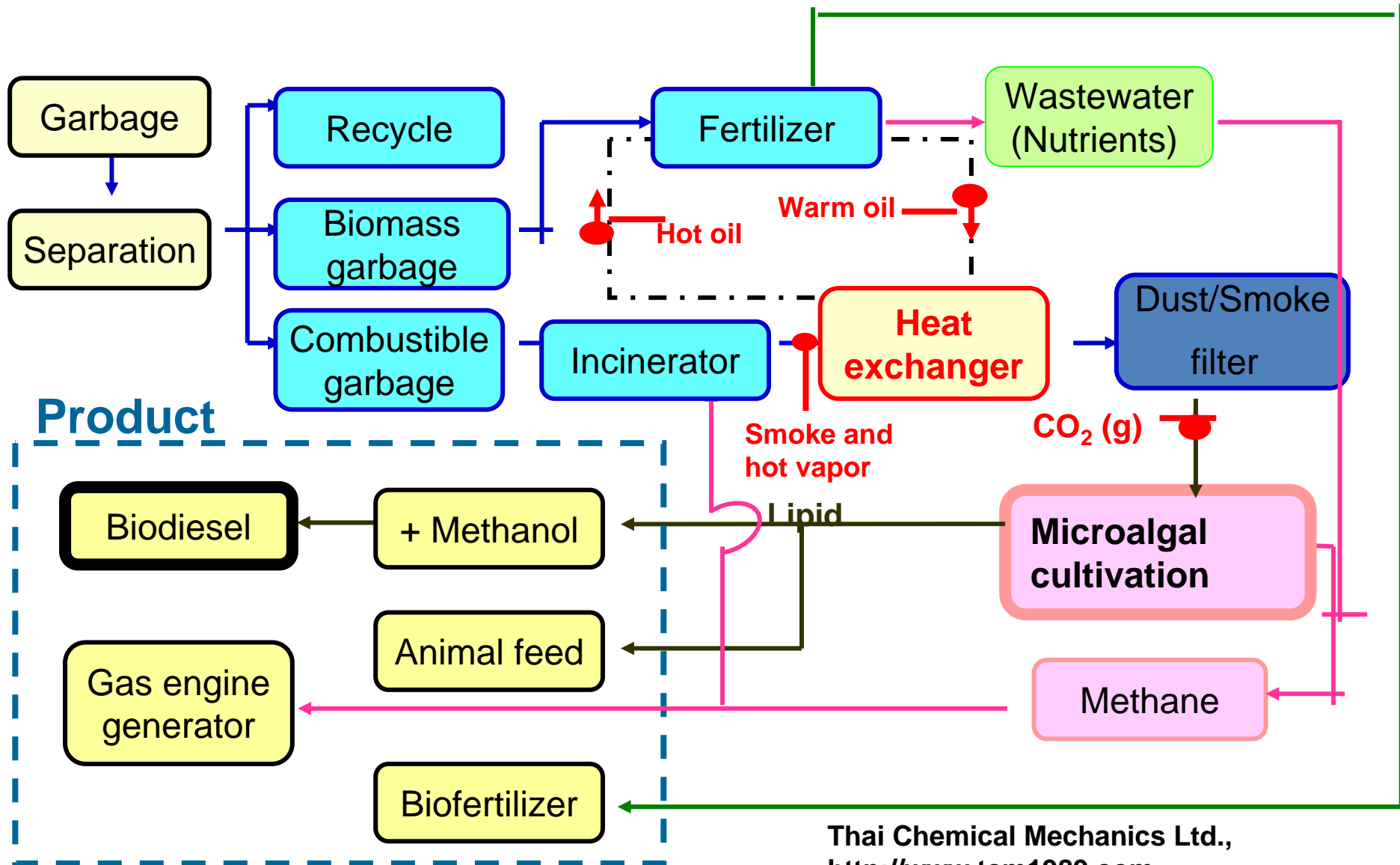
# Harvesting process



Mixing of wastewater and fertilizers



# Garbage to Bio-Fertilizer & Energy Next day



# Ongoing Projects

1. A study of signal transduction mechanic in response to temperature change of *S. platensis* C1 by using proteomics approach; phase2
2. Identification of regulatory elements and regulatory proteins of *Spirulina-des* D gene in response to temperature stress
3. Development of a gene transfer system in *S. platensis* C1 through circumvention of the restriction enzyme
4. *Spirulina* genome
5. Microalgal oil development
6. Optimization of nutrients for a low-cost medium used for *Spirulina* mass production
7. Phycocyanin production from *Spirulina* using membrane technology in pilot scale
8. Bioactive compounds from *Spirulina*
9. Effect of salt stress on lipid biosynthesis in *Spirulina* regulation of phycocyanin biosynthesis in *Spirulina* under high temperature (Microarray)

# *Spirulina* cultivation in Thailand



# Commercial plant near Bangkok



# Boonsom Farm, Chiangmai

- Food grade
- Total production capacity 60 ton/y
- Current production 20 ton/y



# Aquaculture: *Chaetocoeros* production plant



# Microalgal Researches in SE Asia

Most of the research topics are similar to the researches in Thailand (diversity, taxonomy, wastewater utilization)

There are not many commercial plants in the region. Most of the commercial plants are located in China, Taiwan and India.

*Spirulina* production plant (Dainippon Inks) moved their production from Thailand to Hainan, China in the year 2008.

- Recently, the Philippines government (Department of Science and Technology, Philippines Council for Aquatic and Marine Resources) granted a funding to the University of Philippines Los Banos to study microalgal oil.
- Indonesia, Bogor Institute of Technology also studies the potential of using microalgae oil and mentioned that Indonesia could be the 'Middle East' of algae oil.

Thank you for your attention

**Comparison of properties of microagal oil, conventional diesel fuel, and ASTM biodiesel standard (Xu, H. et al., 2006)**

Properties	Biodiesel from microalgae oil	Diesel Oil	ASTM biodiesel standard
Density (kg L <sup>-1</sup> )	0.864	0.838	0.84-0.90
Viscosity (mm <sup>-2</sup> s <sup>-1</sup> , cSt at 40°C)	5.2	1.9-4.1	3.5-5.0
Flash point (°C)	115	75	Min 100
Solidifying point (°C)	-12	-50 to 10	-
Cold filter plugging point (°C)	-11	-3.0 (max -6.7)	Summer max 0 Winter max <-15
Acid value (mg KOH g <sup>-1</sup> )	0.374	Max 0.5	Max 0.5
Heating value (MJ kg <sup>-1</sup> )	41	40-45	-
H/C ratio	1.81	1.81	-

- Taxonomy (Chiangmai, XXX)

Conference

- EU-Southeast Asia Expert Meeting on 2<sup>nd</sup> Generation Biofuels: Identifying Opportunities for Collaboration

(September 8-9, 2009 – Bangkok, Thailand

- (September 10-11, 2009 – Vietnam, Thailand

- Workshop in Advanced in Algal Mass Cultivation

- (September 28 – October 2, 2009 – KMUTT, Bangkok, Thailand

## Oil content of some microalgae

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Microalga	Oil content (% dry wt)
<i>Botryococcus braunii</i>	25-75
<i>Chlorella</i> sp.	28-32
<i>Cryptothecodinium cohnii</i>	20
<i>Cylindrotheca</i> sp.	16-37
<i>Dunaliella primolecta</i>	23
<i>Isochrysis</i> sp.	25-33
<i>Monallanthus salina</i>	>20
<i>Nannochloris</i> sp.	20-35
<i>Nannochloropsis</i> sp.	31-68
<i>Neochloris oleoabundans</i>	35-54
<i>Nitzschia</i> sp.	45-47
<i>Phaeodactylum tricornutum</i>	20-30
<i>Schizochytrium</i> sp.	50-77
<i>Tetraselmis sueia</i>	15-23

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**Characteristics of treated waste water from pig farm before and after treating with *Spirulina* cultivation**

Index	Maximum-Permitted-Value *		Effluent of UASB**	<i>Spirulina</i> treated wastewater
	Standard-a (large farm)	Standard-b (medium farm)		
pH	5.5-9	5.5-9	7.94±0.01	9.8 ±0.2
Total alkalinity (mg/L)	N/A	N/A	1,040 ±28	3,352 ±1,438
Ammonia (mg/L)	N/A	N/A	144 ±16	<5
Nitrate (mg/L)	N/A	N/A	19.9 ±0.8	49 ±48
Phosphorus (mg/L)	N/A	N/A	88 ±0	15 ±5
TKN (mg/L)	120	200	150	4.76
BOD (mg/L)	60	100	30	-
COD (mg/L)	300	400	155	27
Suspended solid (mg/L)	150	200	96 ±8	16 ±6
Total solid (mg/L)	N/A	N/A	1.28 ±0.02	8.6 ±4.5

\*Standard for effluent from pig farm issued by the Ministry of Natural Resources and Environment, Thailand

\* Effluent of UASB before treating with *Spirulina*