

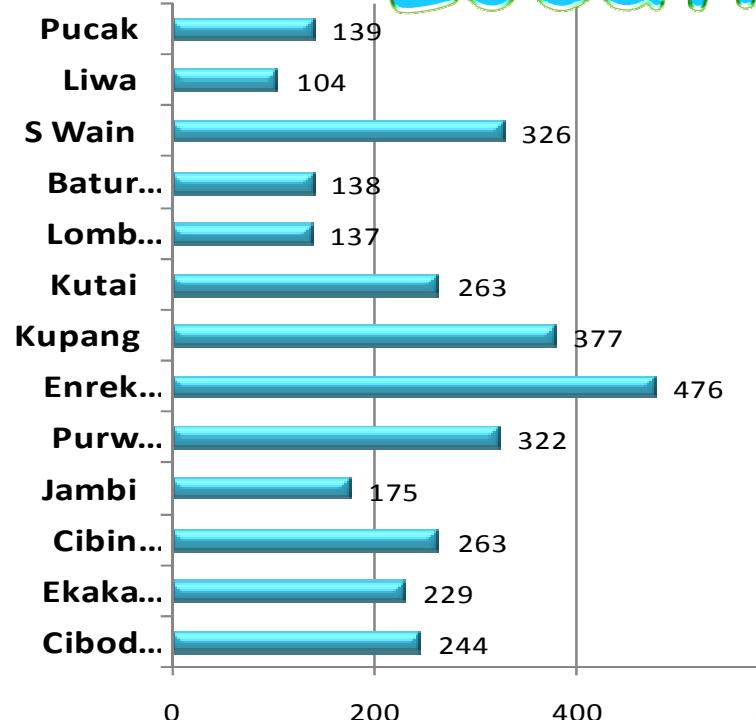
## **Actinobacteria:**

**Currently, order Actinomycetales consist of 13 suborders, 42 families, and 198 genera; while order Rubrobacterales consist of 5 families (Zhi et al., 2009).**

**Indonesian Actinobacteria:  
12 suborders, 27 families, and  
65 genera for 3,193 isolates**



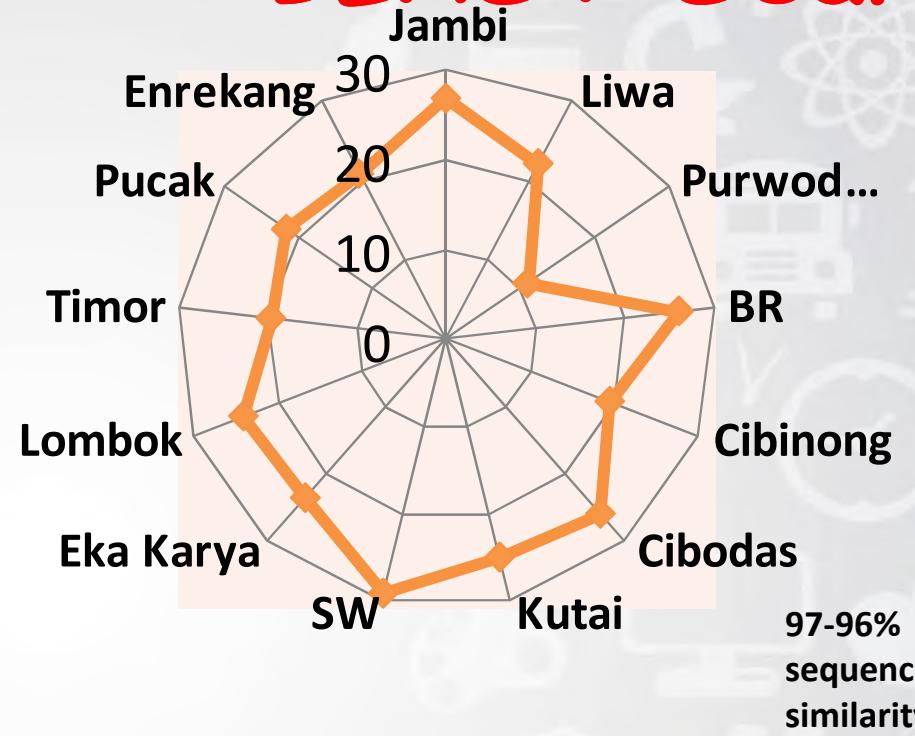
# Location



# Source

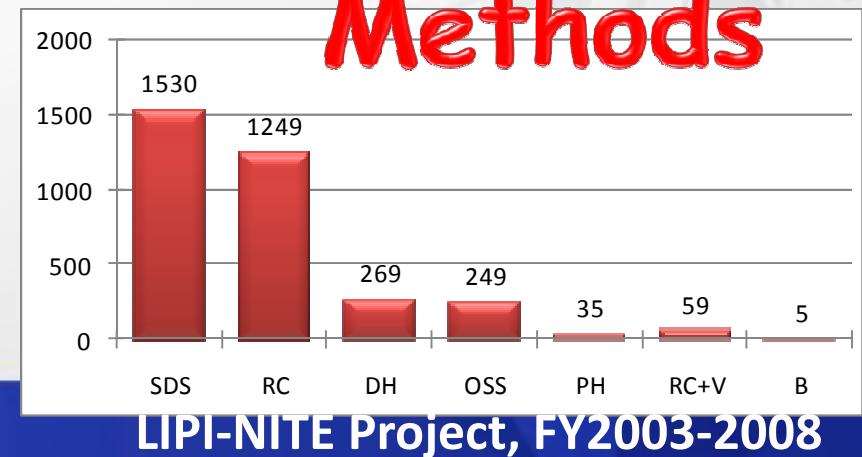


# BLAST Search



97-96%  
sequence  
similarity

# Methods



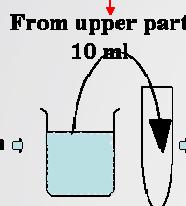
# Isolation Methods



RC

## Rehydration and Centrifugation method

1/10 Soil extract  
0.01 mM Phosphate (KK) buffer 50 ml  
+  
Soil 0.5 g



From upper part  
10 ml

**Do not move!**

Carry out near centrifugation

Centrifugation  
20 min,  
3000 g

leave at rest  
15 min

1 ml

Dilution

1 ml

From upper part  
2 ml

10<sup>-3</sup> 10<sup>-4</sup>

Time is exact (<+5 min)  
At first, 2 ml of supernatant  
are calmly transferred to new  
tubes

RV

## SDS-YE method

Soil  
Sample  
Air-drying

Soil water  
suspension  
10<sup>-1</sup>

10<sup>-2</sup>  
YE 6% + SDS 0.05% in  
P-buffer (0.005 M,  
pH 7.0), 40°C, 20 min

Dilution  
with water

Inocula  
(10<sup>-5</sup>, 0.1-0.2 ml)

HV agar +  
nalidixic acid 20 mg/l

Time is exact (<+5 min)

Soil  
Sample  
1 g

DW 10 ml

YE-SDS 9 ml

DW 9 ml

DW 9 ml

DW 9 ml

Dilution

1 ml

1 ml

1 ml

1 ml

1 ml

At first, every  
samples are diluted  
up to 10<sup>-3</sup>, and then  
are diluted,  
respectively.

10<sup>-2</sup>

10<sup>-3</sup>

10<sup>-4</sup>

10<sup>-5</sup>



[www.lipi.go.id](http://www.lipi.go.id)

## Dry Heating (DH) method

DH

Spread out the soils directly

1 g of  
sieved and air-  
dried soil  
samples

Dry-heating  
120°C, 1 h

HV agar + nalidixic  
acid 20 mg/l

## Phenol method

PH

Soil sample  
Air-drying  
Dry heat 120°C, 1 h  
(same sample with DH)

Soil-water  
suspension 10<sup>-1</sup>

P-buffer contained  
1.5% phenol  
30°C, 30min 10<sup>-2</sup>

Inocula  
(10<sup>-3</sup>, 10<sup>-4</sup>, 0.2 ml)

Dilution with  
water

HV-agar +  
Nalidixic acid 10 mg,  
Cycloheximide 50 mg,  
Kabicidine 0.75 mg/l

## Isolation method of lipophilic actinomycetes - Oil Separation (OS) method -

OS

0.5 g of soil

5ml of  
water

Separation of  
lipophilic Actinomycetes

Mix for 5 min.  
5 ml of olive oil  
5 ml of water

10<sup>-1</sup>

10<sup>1</sup>

10<sup>1</sup>

oil layer  
60°C, 30 min.

HV agar + Nalidixic acid (10 mg/l) and  
Chlortetracycline (50 mg/l)

LIPI-NITE Project, FY2003-2008

# Diversity of Indonesian Actinobacteria

No.	Suborder	Litter	Soil	Total	%
<b>Order Actinomycetales</b>					
1	<i>Catenulisporineae</i>	0	2	2	0.06%
2	<i>Corynebacterineae</i>	1	105	106	3.32%
3	<i>Frankineae</i>	14	26	40	1.25%
4	<i>Glycomycineae</i>	1	0	1	0.03%
5	<i>Kineosporiineae</i>	83	9	92	2.88%
6	<i>Micrococcineae</i>	4	22	26	0.81%
7	<i>Micromonosporineae</i>	324	392	716	22.42%
8	<i>Propionibacterineae</i>	3	40	43	1.35%
9	<i>Pseudonocardineae</i>	24	49	73	2.29%
10	<i>Streptomycineae</i>	184	1702	1886	59.07%
11	<i>Streptosporangineae</i>	0	207	207	6.48%
<b>Order Rubrobacterales</b>					
12	<i>Patulibacteraceae</i>	1	0	1	0.03%
		638	2558	3193	100%



# Description of New Taxa

- Otoguro, M., Ratnakomala, S., Lestari, Y., Hastuti, R. D., Triana, E., Widyastuti, Y., & Ando, K. 2009. *Streptomyces baliensis* sp. nov., isolated from Balinese soil. Int. J. Syst. Evol. Microbiol., 59: 2158-2161
  - Yamamura, Y., Lisdiyanti, P., Ridwan, R., Ratnakomala, S., Sarawati, R., Lestari, Y., Triana, E., Kartina, G., Widyastuti, Y., & Ando, K. 2010. *Dietzia timorensis* sp. nov., isolated from soil. Int. J. Syst. Evol. Microbiol., 60: 451- 5 454
  - Lisdiyanti, P., Otoguro, M., Ratnakomala, S., Lestari, Y., Hastuti, R. D., Triana, E., Ando K., & Widyastuti, Y. 2010. *Actinokineospora baliensis* sp. nov., *Actinokineospora cibodasensis* sp. nov., and *Actinokineospora cianjurensis* sp. nov. isolated from Indonesia. Int. J. Syst. Evol. Microbiol., in press.
  - Otoguro, M., Yamamura, H., Tamura, T., Irzaldi, R., Ratnakomala, S., Ridwan, R., Kartina, G., Triana, E., Nurkanto, A., Lestari, Y., Lisdiyanti, P., Widyastuti, P., & Ando, K. 2011. *Actinophytocola timorensis* sp. nov. and *Actinophytocola corallina* sp. nov., isolated from soil in Indonesia





# InaCC (Indonesian Culture Collection)

InaCC Inauguration Ceremony  
11 September 2014



Indonesian Culture Collection (InaCC)  
Pusat Penelitian Biologi  
Lembaga Ilmu Pengetahuan Indonesia

Document InaCC



# InaCC Facilities

- **2 Storeys Laboratory Building, 2800 M<sup>2</sup>**



Multimode Microplate reader



High Performance Liquid Chromatography

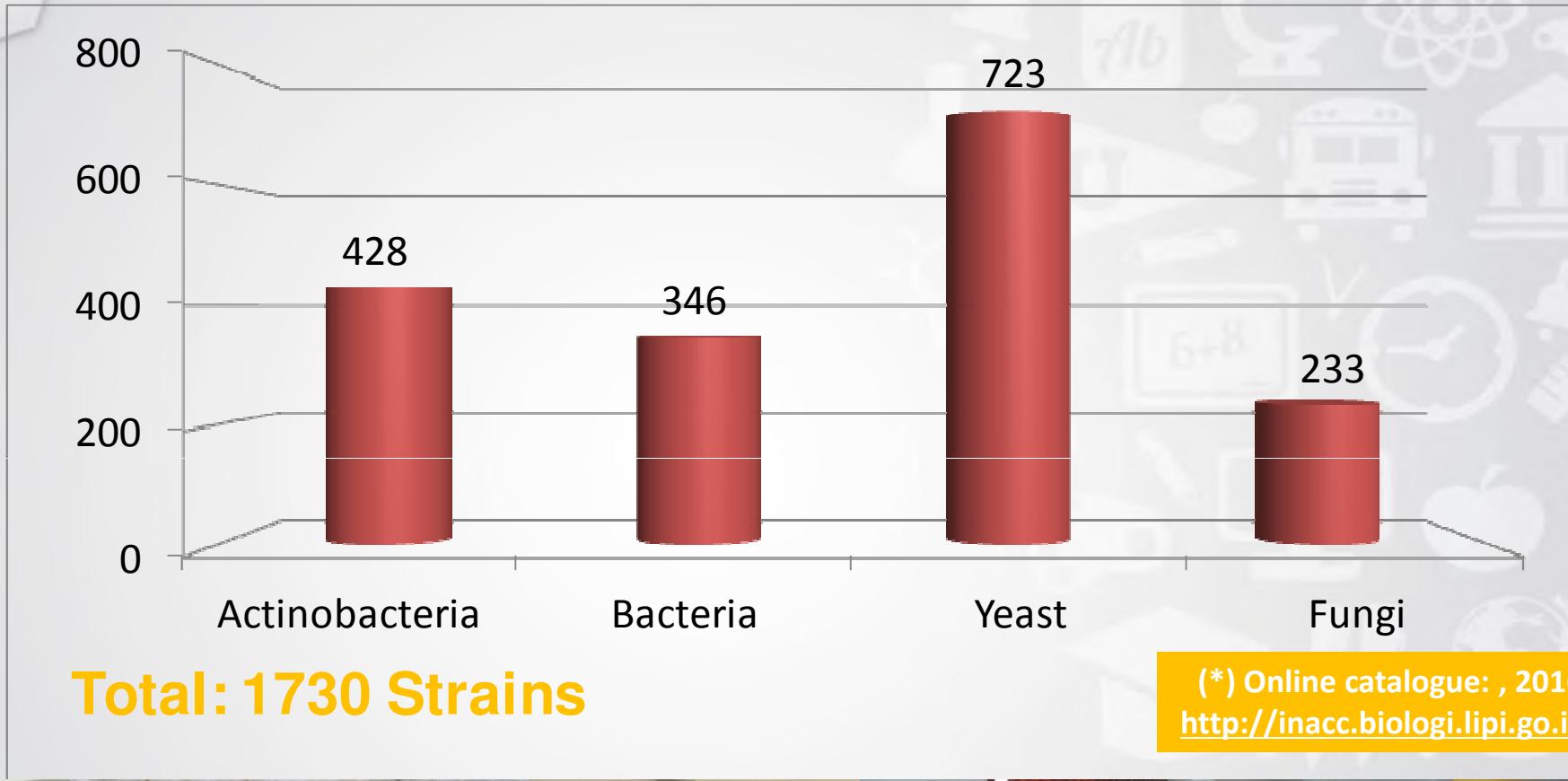


Matrix Assisted Laser Desorption/Ionizing Time Of Flight – Mass Spectrometry (MALDI TOF – MS)





# InaCC Holdings\*



**Total: 1730 Strains**

(\*) Online catalogue: , 2016  
<http://inacc.biologi.lipi.go.id/>



Document InaCC



# Herbarium Bogoriense

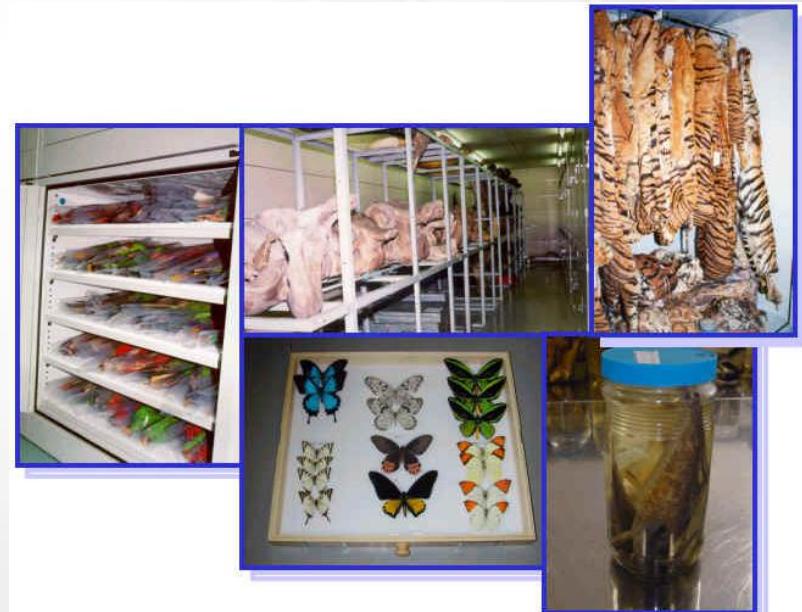
## Botany Collection

- > 2.000.000 specimens :
- Wet collections
- Dry Collections
- Karpologi
- Fossil
- Artefact Etnobotany



# Museum Zoologicum

Takson	Indonesia		Koleksi di MZB
	Minimum	Maksimum	
Mamalia	681	800	450 (66%)
Burung	1.540	1.600	1.200 (78%)
Reptilia	600	2.000	340 (57%)
Amphibia	1.000	1.500	150 (10%)
Ikan	7.000	8.500	1.200 (17%)
Mollusca	4.000	6.000	2.400 (60%)
Invertebrata lain	5.000	10.000	700 (14%)
Serangga	1.000.000	5.000.000	10.000 (1%)





**4th JASTIP Symposium**  
**"Biomass to Energy, Chemicals, and Functional Materials"**  
**3rd and 4th July 2017 NSTDA, Thailand**

**SATREPS Project: Development of Internationally Standardized Microbial Resource Center to Promote Life Science Research and Biotechnology**

Ken-ichiro Suzuki\*, Hiroko Kawasaki Biological Resource Center, National Institute of Technology and Evaluation (NBRC) and Witjaksono, Siti N. Projono and Bambang Sunarko Indonesian Institute of Sciences (LIPI) \*Present address: Tokyo Univ. Agriculture

**Purpose of Project**

- Development of Indonesian microbial resources for human health and environmental restoration
- An ex-situ conservation of Indonesian microbial resources through improvement of Indonesian Microbial Collection at RCB-LIPI
- Sustainable utilization of Indonesian microbial resources for improving food and health
- Creating global partnership between culture collection center and stimulating development of biotechnology in Indonesia and Japan

**Output of Project:**

1. InaCC, Indonesian national microbial resource centers, was established in LIPI to collect and preserve microorganisms to promote further study and application.
2. InaCC will contribute to access and use of Indonesian microorganisms in compliance with CBD related laws and regulations.
3. Diversity and potential of Indonesian microorganisms were recognized.
4. More than 2000 strains of various microorganisms, including filamentous fungi, yeasts, microalgae, bacteria, archaea and bacteriophages were registered and preserved in InaCC with their taxonomic information.

**Summary of Research for New Microbes**

- Yeast accumulating lipid in the cells were isolated.
- Yeast utilizing xylose were isolated.
- New actinomycete species were isolated and characterized.
- Endophytic fungi in chinchoane were isolated.
- Ecotrophic fungi for Dipterocarpus and Sumatra pine were isolated.

**Outlines of Project**

RS-3: Univ. Tokyo, RIKEN, Ecological study of soil microorganisms  
RS-4: RIKEN, Health of animals  
RS-2: NITE, Taxonomy and preservation  
RS-1: Indonesian Culture Collection, InaCC Research Center for Biology, LIPI, Microbes characteristic to Indonesia

Discovery of microorganisms with potential for protection of environment and improvement of human life from the diverse natural resources of Indonesia

**Establishment of InaCC**

- Technology was transferred for preservation, quality maintenance and management of microbial resource center
- Cooperative studies were achieved and microorganisms isolated from Indonesian environment were added in InaCC collection.
- InaCC is supplying microbial strains to researchers and industries in compliance with Indonesian laws and regulations
- InaCC will support international cooperation including biological material transfer

InaCC was certified by ISO9001:2008 for Quality management

Research Subject	Microbes	Collection	
		InaCC	NBRC
RS-1-A	Fungi	376	42
	Bacteria	604	51
RS-1-B	Fungi	12	-
	Bacteria	16	2
RS-1-C	Actinomycetes	487	44
	Bacteria	42	-
RS-1-D	Bacteria	228	37
	Archaea	70	16
RS-1-E	Bacteriophages	9	1
	Microalgae	89	18
RS-1-F	Bacteria	349	41
	Fungi	102	10
RS-1-G	Bacteria	97	1
	Bacteria	94	-
RS-1-H	Actinomycetes	1	-
	Microalgae	342	10

**Acknowledgment:**

SATREPS Colleagues of NBRC / NITE and other Japanese members  
SATREPS Colleagues of RCB-LIPI and other Indonesian members

This work was partly supported by the Science and Technology Research Partnership for Sustainable Development (SATREPS), which is a research program structured as a collaboration of the Japan Science and Technology Agency (JST) and the Japan International Cooperation Agency (JICA).

InaCC (Building LIPI (3,500 m<sup>2</sup>)



# Linking SATREPS

1. April 2011-March 2016 SATREPS PROJECT: Development of Internationally Standardized Microbial Culture Collection in Indonesia LIPI-JICA-JST-NBRC (NITE), Japan
2. April 2013-March 2018 SATREPS PROJECT: Innovative Bio-production in Indonesia (ibiol): Integrated Bio-refinery Strategy to Promote Biomass Utilization using Super-microbes for Fuels and Chemicals Production LIPI-JICA-JST-Kobe University
3. April 2014-March 2019 SATREPS PROJECT: Searching Lead Compounds of Anti-malarial and Anti-amebic Agents by Utilizing Diversity of Indonesian Bioresources BPPT-LIPI-JICA-JST-Tsukuba University
4. April 2015-March 2020 SATREPS PROJECT: Revegetation of alang-alang (*Imperata cylindrica*) field combined with sustainable production and utilization of biomass (for energy solution), LIPI-JICA-JST-Kyoto University



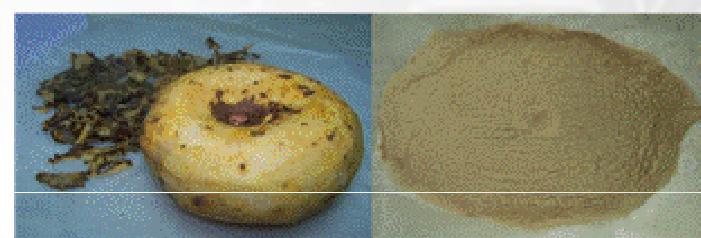
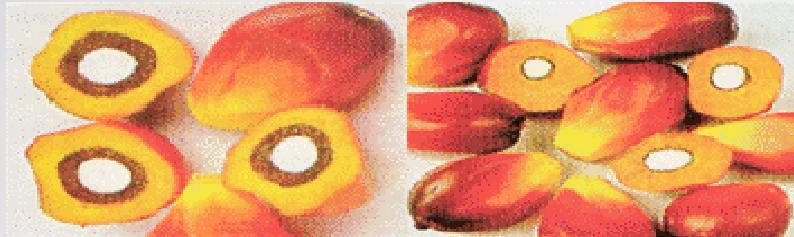


# **Screening & Cloning of Microbial Collection for Biorefinery Development (2011-present)**

- ❖ Research Center for Biotechnology LIPI
- ❖ JSPS-LIPI Bilateral Project (FY2011-2013)
- ❖ Satreps Biorefinery (FY2013-2018)
- ❖ Center of Excellent, Ministry of Research, Technology, and Higher Education (December 2016)



# Research on Utilization of Hetero-mannan biomass



Palm kernel cake : Galactomannan

JSPS-LIPI Bilateral Project  
(RC-Biotechnology - Kobe Univ.)

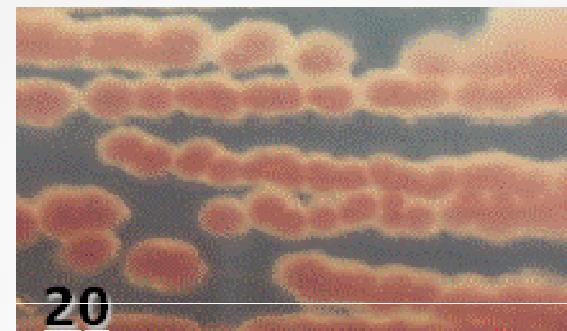
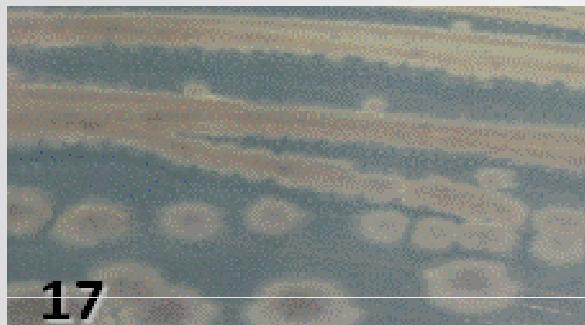
Porang potato : Glucomannan

Competitive Project  
(LIPI)

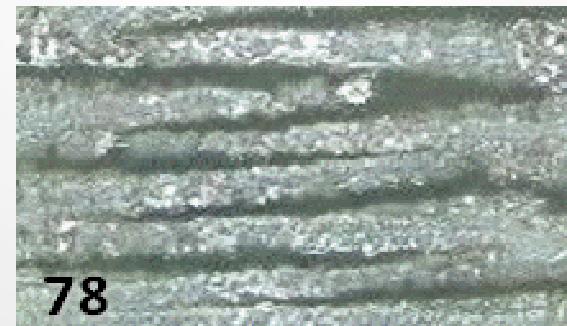
## Main Activity :

Utilization of Palm kernel cake to produce bioethanol and Porang potato to produce oligosaccharides enzymatically (using mannanase, mannosidase from actinobacteria)

# Several types of mannolytic microbes from Actinobacteria



6 positive mannolytic bacteria, selected from 500 actinomycetes BTCC





# Satreps Biorefinery Activities

## Activity

### 2.1. Production of lignocellulose hydrolyzing enzymes

2.1.1. Screening and optimizing of cellulase and hemicellulase producing microbes from Indonesia Culture Collection (InaCC) and Biotechnology Culture Collection (BTCC)

2.1.2. Cloning of cellulase and hemicellulase genes from microbes in InaCC and BTCC

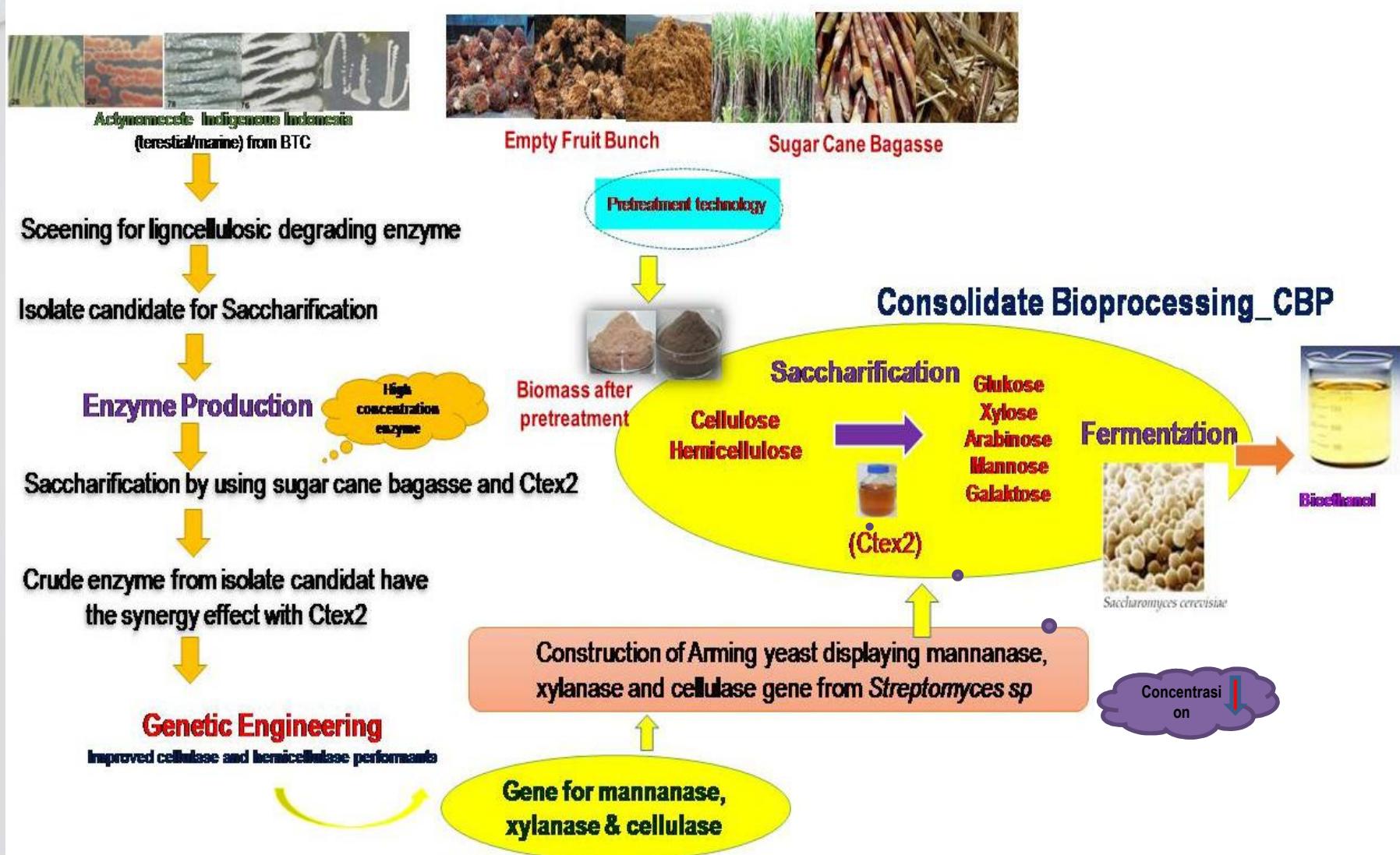
2.1.3. Construction of the expression system for enzyme production

2.1.4. Purification and characterization of recombinant enzymes

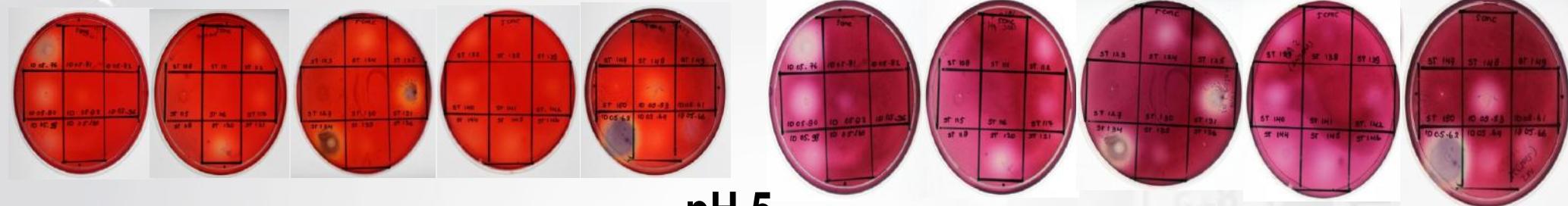
### 2.2. Evaluation of lignocellulose hydrolyzing enzymes

2.2.1. Lignocellulose degradation by produced enzymes

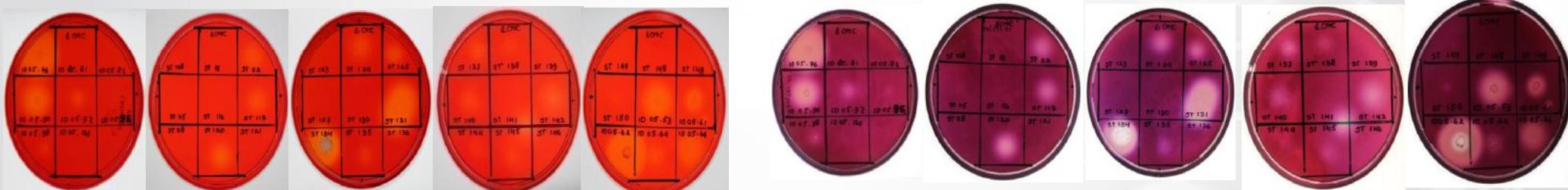
# Strategy for Research



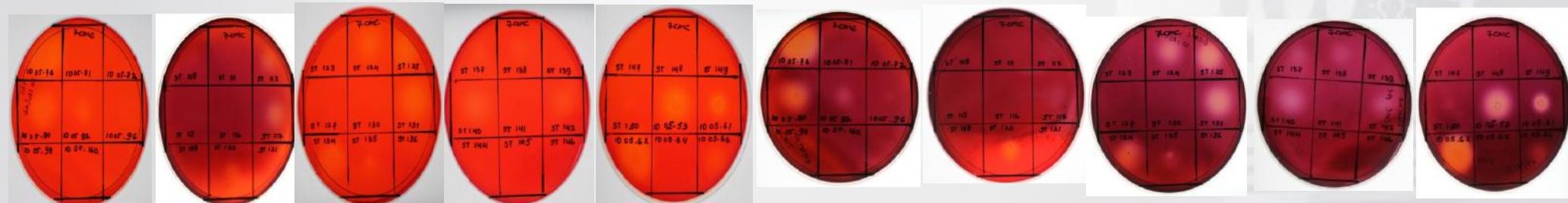
# Result of Congo Red & Acetic acid Analysis of 44 isolates on CMC plate medium, pH 5, 6 and 7



pH 5



pH 6



pH 7

Isolate with diameter clear zone > 1.5 cm : isolates No. ID 05 76, S T131, ST134, ID05 53, ID 0562

ORIGINAL ARTICLE

Open Access



# Mannan endo-1,4- $\beta$ -mannosidase from *Kitasatospora* sp. isolated in Indonesia and its potential for production of mannooligosaccharides from mannan polymers

Nanik Rahmani<sup>1</sup>, Norimasa Kashiwagi<sup>2</sup>, JaeMin Lee<sup>3</sup>, Satoko Niimi-Nakamura<sup>3</sup>, Hana Matsumoto<sup>3</sup>, Prihardi Kahar<sup>3</sup>, Puspita Lisdiyanti<sup>1</sup>, Yopi<sup>1</sup>, Bambang Prasetya<sup>1</sup>, Chiaki Ogino<sup>3\*</sup> and Akihiko Kondo<sup>2,4</sup>

## Abstract

Mannan endo-1,4- $\beta$ -mannosidase (commonly known as  $\beta$ -mannanase) catalyzes a random cleavage of the  $\beta$ -D-1,4-mannopyranosyl linkage in mannan polymers. The enzyme has been utilized in biofuel production from lignocellulose biomass, as well as in production of mannooligosaccharides (MOS) for applications in feed and food industries. We aimed to obtain a  $\beta$ -mannanase, for such mannan polymer utilization, from actinomycetes strains isolated in Indonesia. Strains exhibiting high mannanase activity were screened, and one strain belonging to the genus *Kitasatospora* was selected. We obtained a  $\beta$ -mannanase from this strain, and an amino acid sequence of this *Kitasatospora*  $\beta$ -mannanase showed a 58–71% similarity with the amino acid sequences of *Streptomyces*  $\beta$ -mannanases. The *Kitasatospora*  $\beta$ -mannanase showed a significant level of activity (944 U/mg) against locust bean gum (0.5% w/v) and a potential for oligosaccharide production from various mannan polymers. The  $\beta$ -mannanase might be beneficial particularly in the enzymatic production of MOS for applications of mannan utilization.

**Keywords:** Mannan endo-1,4- $\beta$ -mannosidase, Mannooligosaccharides (MOS), Screening, *Kitasatospora* sp., *Streptomyces lividans* 1326