

# Soil Biotechnology in Thailand

## Introduction

Soil microorganisms are organisms that live in soil and play critical roles. To begin with, soil fertility, such as microbial decomposing organic matter, produces extracellular enzymes degrading organic matter, allowing soil property to be improved. Some nutrients are recycled into the plant. Essentially, microbial conversion of inorganic compounds plays a role in deforming minerals in the soil that are no longer useful to plants. Nitrogen-fixing bacteria are organisms that can convert nitrogen gas in the atmosphere into nitrogen compounds that plants can use directly. Mycorrhiza fungi will help plants uptake more nutrients, particularly phosphorus.

The production of hormones by microorganisms accelerates the development, flowering, and fruiting of plants as well as the germination of seeds and root crops. Microorganisms that can manufacture organic acids and antibiotics to inhibit plant pathogens that cause plant disease or microorganisms that can produce enzymes like chitinase and ligninase to break down plant pathogen cell walls are examples of microbially controlled plant pathogens. The Land Development Department has conducted research and created microbial products for plant production generated from soil microorganisms. The product's name is Microbial Activator LDD. Currently, there are three types of microbial activator LDD products, each containing ten products. These three categories are increased nutrients and plant hormones, microbial pest controlling, and microbial for environment use.

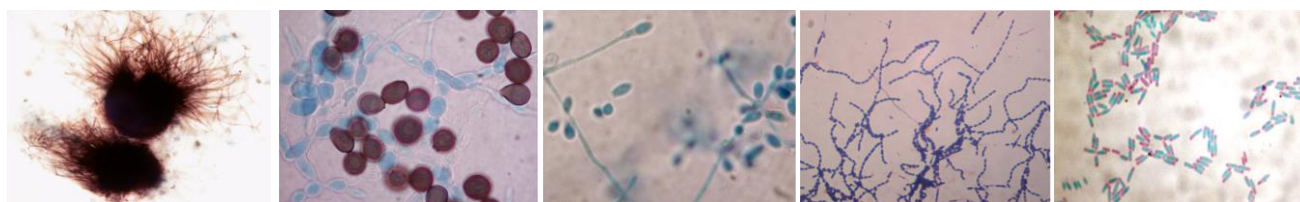


## 1. Microbial products for soil improvement, increasing plant nutrients and plant growth hormones

There are 6 products in these group namely activator Super LDD 1 for composting, Microbial activator Super LDD 2 for bio-extract production, Super LDD 9 for increasing available phosphate in acid and sulfate acid soil, LDD 11 for increasing biomass and plant nutrient of Leguminosae green manure (*Sesbania rostrata*, *Crotalaria juncea* L. and *Canavalia ensiformis* L.) bio-fertilizer LDD 12 and LDD 13 mycorrhiza for maize production.

### 1.1 Microbial activator Super LDD 1 for composting

Microbial activator Super LDD 1 is a group of high efficient microorganisms to decompose crop residue and others organic materials which consist of hardly decompose component such as cellulose, lipid and lignin for making the compost. Microbial activator Super LDD 1 consist of cellulolytic fungi (*Corynascus verrucosus*, *Scytalidium thermophilum*, *Chaetomium thermophilum* and *Scopulariopsis breviacaulis*) cellulolytic actinomycetes (2 strains of *Streptomyces*) and Lipid degrading bacteria (2 strain of *Bacillus* sp.)



*Chaetomium thermophilum*

*Scytalidium thermophilum*

*Corynascus verrucosus*

*Bacillus* sp.

*Streptomyces* sp.

### Raw material proportion and composting procedure

Raw material proportion for making 1 ton of compost

- Plant residue (dry weight)                    1,000    kg
- Animal manure                                    200     kg
- Urea    2        kg (or bio-extract from fish 9 liters)
- Microbial Activator Super LDD 1            1        package (100 g)



## Composting procedure



1) Mixing microbial activator Super LDD 1 in 20 liters of water and stir for 10-15 minutes.



2) Separating of material for 3 layers, the first layer is spread plant residue as the base of pile has width range 2 meters, 3 meters of length with of first layer 50 cm. Watering waste evenly as each layer is completed.



3) Top with animal manure and spread out into an even layer.



4) Dressing with urea or bio-extract from fish on animal manure layer.



5) Distribute microbial Suspension over the compost pile which finish the first layer. Procedure these steps as the second and third layer.



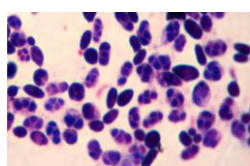
6) Top of heap are covered with a layer of soil / plastic/ coconut leaves to help retain moisture content. layer.

### Application rate of compost

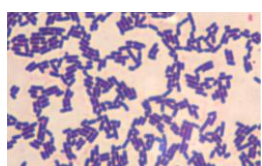
|                  |   |
|------------------|---|
| Rice:            | 12.5 ton/ha, broadcast throughout planting area.  |
| Field crop:      | 12.5 ton/ha, banding along the row of plant.  |
| Vegetable:       | 25 ton/ha, broadcast throughout planting area.  |
| Fruit tree:      | 20- 50 kg/plant, placement compost into the groove and cover with soil.                                       |
| Flowering plant: | cut flower plant use 12.5 ton/ha and flowering tree use 5-10 kg/plant, Broadcasting throughout planting area. |

### 1.2 Utilization of Microbial activator Super LDD 2 for produce bio-extract

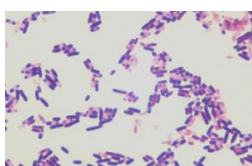
Microbial activator Super LDD 2 is a group of high efficient microorganisms which can activate fermentation and digestion process of organic waste such as fresh and succulent from fruit, vegetable, fish and snail by effective microorganisms in facultative anaerobic condition. The components of bio-extract are plant growth hormones (auxin, gibberillin, cytokinin), amino acid, humic acid, organic acid, vitamin and minerals. Microbial activator Super LDD 2 is composed of 5 different microbial stains.



*Pichai membranifaciens*



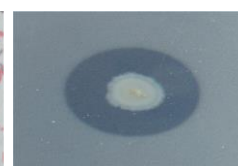
*Lactobacillus fermentum*



*Bacillus megaterium*



*Bacillus subtilis*



*Burkholderia unamae*

### 1) Raw materials to produce bio-extract

#### 1.1) Formula of bio-extract from fruit and vegetable (Fermentation time: 7 days)



Fresh and succulent of fruit or vegetable residue 40 kg








Molasses 10 kg  
Water 10 liters



Microbial activator Super LDD 2  
1 package (25g)



## 1.2) Formula of bio-extract from fish and snail (Fermentation time: 15-20 days)

|   |   |   |  |   |
|---|---|---|--|---|
|  |  |  |  |  |
| Fresh and succulent of fish, snail 30 kg  | Fresh and succulent of fruit residue (pine apple) 10 kg                           | Molasses 10 kg<br>Water 10 liters   | Microbial activator Super LDD 2<br>1 package (25g)                                 |   |

### 2) Step to produce bio-extract



1) Mix molasses with water thoroughly in the mixing tank.



2) Suspend microbial activator Super LDD 2 1 pack in molasses or other sugar and stir continuously for 5 minutes.



3) Put raw material, witch chop into small pieces in mixing tank.



4) And mix together.



5) Close a cover and put the tank under shady area, stir these substances every day.

### 3) Consideration of completely fermentation process

- Reduction of microbial growth.
- Reduction of CO<sub>2</sub> bubble.
- Reduction of alcoholic smell.
- Dropping of sour smell.
- Dropping of pH down to 3-4.

### 4) Utilization and application rate of bio-extract

#### 1. Rice

- Soaking seed: mix bio-extract 40 milliliters in water 20 liters, soaking seed for 12 hrs., taken up 1 day and then broadcast.
- Incorporation of rice stubble: mix bio-extract 31.25 liters in water 625 liters, pour throughout planting area 1 ha and ferment 15-10days.
- During growth stage: mix bio-extract 12 tablespoons in water 60 liters, spray at 30, 50 and 60 day during growing stage.

2. Field crop: mix bio-extract 3.75 liters in water 1,250 liters spray every 10 days before flowering stage in area 1 ha.

3. Vegetable and flower: mix bio-extract 37.5 tablespoons in water 625 liters spray every 10 days in area 1 ha.

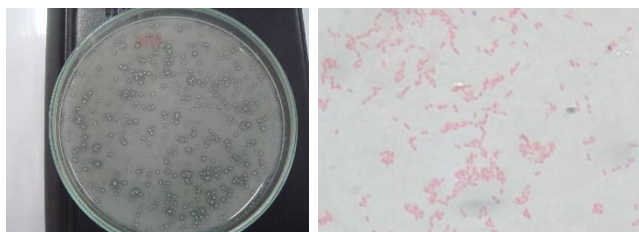
4. Fruit tree: mix bio-extract 20 tablespoons in water 100 liters spray every 1 month before flowering stage.



### 1.3 Microbial Super LDD 9 for increasing available phosphate in acid and acid sulfate soil by

Microbial Super LDD 9 is phosphate solubilizing bacteria, capable of dissolve phosphorus from insoluble compounds to available form in acid soil and acid sulfate soil (pH less than 4). Microbial Super LDD 9 is composed of *Burkholderia* sp.





*Burkholderia* sp.

## 1) Raw material for Microbial Super LDD 9 cultivation



Compost 300 kg



Rice 3 kg

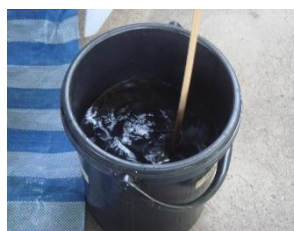


Microbial Super LDD 9  
1 package

## 2) Step to microbial super LDD 9 cultivate



1. Mix compost with  
rice bran



2. Suspend microbial  
activator Super LDD 9  
in water, stir continuously  
for 5 minutes.



3. And pour microbial  
super LDD 9 solution into  
material



4. Mix together and  
maintain moisture content  
approximately 70%.



5. Such materials are  
piled as rectangular



6. Top of pile is  
covered and maintains



### 3) Utilization and application rate of Microbial Super LDD 9

- Rice, field crop, vegetable and ornamental plant: 625 kg/ha, broadcast throughout planting area or banding along the row of plant.

- Fruit tree: 3 kg/plant, placement into the hole and cover with soil or application around the bush.

#### 1.4 Microbial for green manure LDD 11

Microbial for green manure LDD 11 is a group of co - inoculation between nitrogen fixing bacteria (Rhizobium) and phosphate solubilizing bacteria produce organic acid to dissolve inorganic phosphorus complex to available from: *Burkholderia* sp. for increase biomass and nutrient content of leguminosae green manure (*Sesbania rostrata* and *Crotalaria juncea*).



Rhizobium



Phosphate solubilizing bacteria

#### Raw material to microbial activator for green manure LDD 11 cultivation

|                            |     |                 |
|----------------------------|-----|-----------------|
| Compost                    | 100 | kg              |
| Rice bran                  | 1   | kg              |
| Microbial activator LDD 11 | 1   | package (100 g) |





## Procedure to cultivate



1. Mixing microbial activator for green manure in LDD 11 and rice bran in water 5 liters, then 5 minutes stirring



2. Pour suspends solution of microbial activator for green manure in LDD 11 into compost pile and mix together. Such materials are piled in rectangular with 50 cm height under shady area. Top of pile are covered and moisture content about 60-70 % throughout 4 days.

### Application rate of LDD 11 cultivation (*Sesbania rostrata* and *Crotalaria juncea*)

- Broadcasting throughout planting area banding along the row of plant 625 kg/ha.
- Broadcasting of *Crotalaria juncea* Seeding rate 31.25 kg/ha.
- Seed of *Sesbania rostrata* were soaked in running tap water overnight to break down seed dormancy. Broadcasting seeding rate 31.25 kg/ha.

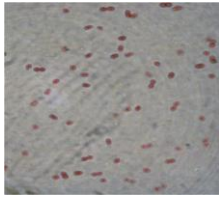
### Advantage

- 1) Increase nitrogen in the soil, moreover it can be nitrogen source in organic agriculture system. Increase fresh weight, dry weight of African Doncha and Sunhemp.
- 2) Increase organic matter, soil fertility, improve physical and chemical including biological properties of soil.
- 3) Increase economic crop yield.

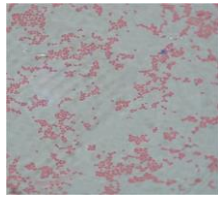


### 1.5 Bio-fertilizer LDD 12

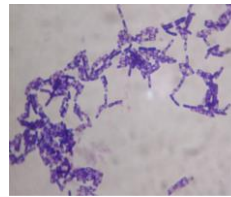
Bio-fertilizer LDD 12 is a group of effective microorganisms that can produce nutrient elements or convert insoluble inorganic compounds into soluble form to increase soil fertility and produce plant growth hormone to improve plant growth. LDD 12 is composed of 4 different of bacteria strains namely Free-living nitrogen fixing bacteria (*Azotobacter tropicalis*), Phosphate solubilizing bacteria (*Burkholderia unamae*), Potassium solubilizing bacteria (*Bacillus subtilis*) and Plant growth promoting rhizobacteria (*Azotobacter chroococcum*).



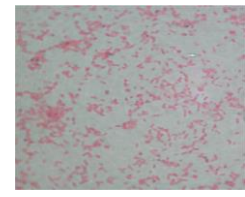
*Azotobacter tropicalis*



*Burkholderia unamae*



*Bacillus subtilis*



*Azotobacter chroococcum*

#### Raw material to biofertilizer LDD 12 cultivation

|                      |     |                 |
|----------------------|-----|-----------------|
| Compost              | 300 | kg              |
| Rice bran            | 3   | kg              |
| Biofertilizer LDD 12 | 1   | package (100 g) |
| Cultivation time     | 4   | days            |

#### Procedure to cultivate LDD 12



1. Mixing biofertilizer LDD12 and rice bran in water 20 liters.



2. Pour solution of biofertilizer LDD 12 into compost and mix together



3. Such materials are piled as rectangle with 50 cm height under shading area. Top of pile is covered and maintained moisture to 60-70% throughout 4 days.

### Utilization and application rate of bio-fertilizer LDD 12

- Rice, field crop, vegetable and ornamental plant: 1,875 kg/ha, broadcast throughout planting area or banding along the row of plant.
- Fruit tree: 5 kg/plant, placement into the hole and cover with soil or application around the bush.

Note: Application rate of chemical fertilizer as decrease 25 % of recommend rate

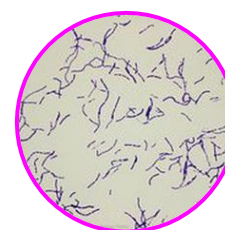
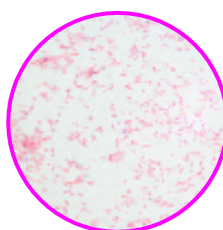
### 1.6 LDD 13 Mycorrhiza for maize

**Mycorrhiza** are a symbiotic association between plant roots and fungi. Their major role is to enhance nutrient and water uptake by the host plant by exploiting a larger volume of soil than roots alone can do.

**LDD 13** is a microbial product which recommend for maize production The 3 major groups of effectives microorganisms of LDD 13 are arbuscular mycorrhizal fungi (AMF), free living nitrogen fixing bacteria and plant growth promoting rhizobacteria. LDD 13.

#### Formulations of LDD 13

- Arbuscular mycorrhiza fungi: *Glomus* sp. and *Acaulospora* sp.
- Free living nitrogen fixing bacteria : *Azotobacter chroococcum*
- Plant growth promoting rhizobacteria : *Bacillus* sp.



### Propagation for AMF spores in LDD 13

propagation of LDD 13 can be conducted by using the pot culture method. Maize are be used as a host plant and usually takes 60 days.

### Material for increasing AMF spores in LDD 13



Sand 1 kg.



Compost 4 kg.



Pot/Plastic bag

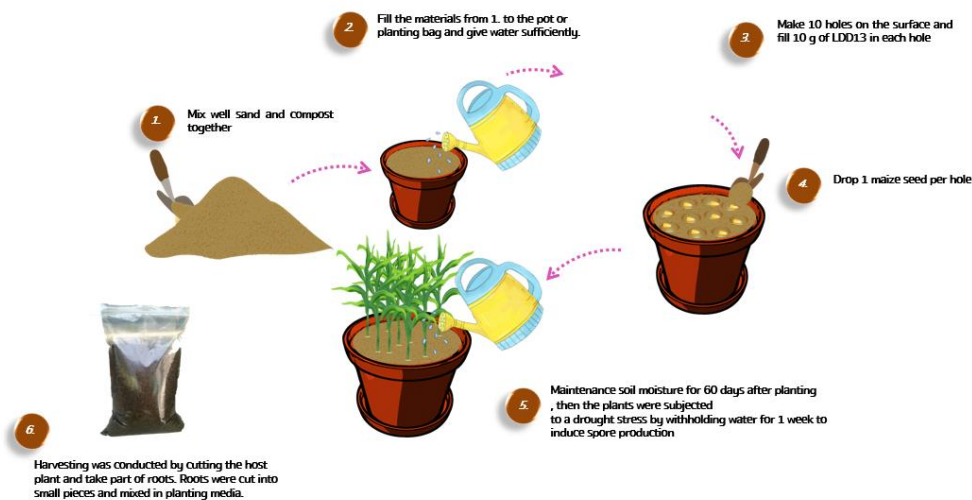


10 Maize seeds



1 pack of LDD 13

### Method for increasing AMF spores in LDD 13



### Application of LDD 13 for maize production

Mycorrhiza is applied to the field with the recommended dose of 20 kg per rai by mixing well with 70 kg compost, Then, 10 g of mixture are put in the hole followed by maize seed.



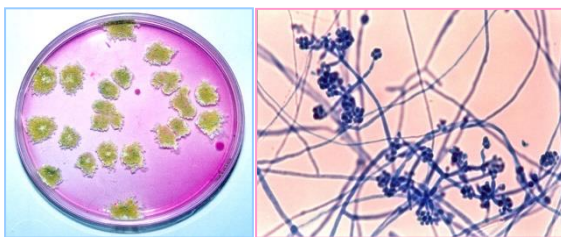
**Benefit**

- Increase absorption of water and plant nutrient
- Plant absorbs higher phosphorus between 20 - 30%
- Decrease using
- 25 - 50% of chemical fertilizer
- Increase 10 - 20% maize yield

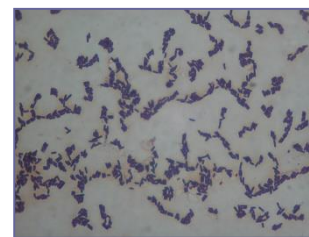
**2. microbial products for biocontrol**

**2.1 microbial activator Super LDD 3**

Super LDD 3 is an antagonistic microorganisms which can control and inhibit soil born plant pathogens both upland and lowland area especially, roots rot disease of economic crops. Super LDD 3 is composed of *Trichoderma* sp. and *Bacillus* sp.



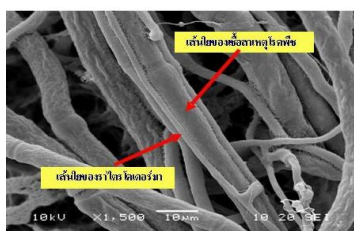
*Trichoderma* sp.



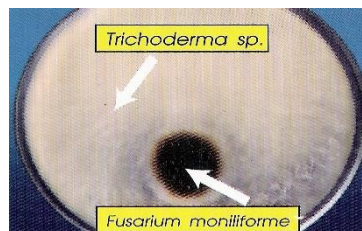
*Bacillus* sp.

**Antagonistic mechanism**

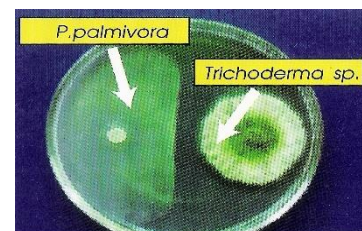
- Ability of nutrient competition and grower than plant pathogen. It is important for limiting disease incidence and severance.
- Directly destruction the hyphae of plant pathogen by producing lytic enzyme to beak cell and uptake protoplasm within hyphae as nutrient source.
- Excretion of antibiotic and toxic substance to inhibit plant pathogen. Antibiotic is microbial toxins which can poison or kill other microorganism and most microbes produce more compounds with antibiotic activity.



Directly destruction plant pathogen



Ability of nutrient competition



Excretion of antibiotic



## Raw material for microbial activator Super LDD 3 cultivation

|                                 |     |                                    |
|---------------------------------|-----|------------------------------------|
| Compost                         | 100 | kg                                 |
| Rice bran                       | 1   | kg (or chicken manual, bat manual) |
| Microbial activator Super LDD 3 | 1   | package (25g)                      |

## Procedure to cultivate



1. Mixing microbial activator Super LDD 3 and rice bran in water 5 liters, 5 minutes stirring.

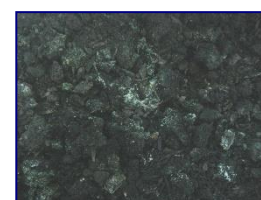


2. Pour solution of Super LDD 3 into those materials and mix together.



3. Such materials are piled as rectangle with 50 cm height under shading area. Top of pile is covered and maintained moisture to 60-70% throughout 7days.

Antagonistic mechanism population of microbial Super LDD 3 are increased by observing white hyphae and green color of Trichoderma spore .mix together and keep under shading area.

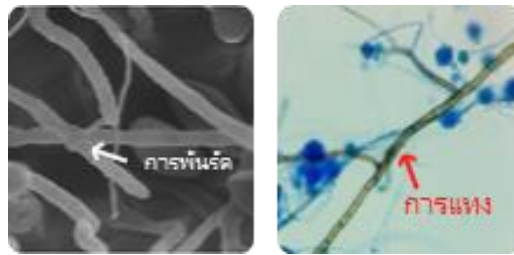


## Utilization and application rate

1. Rice, field crop, vegetable and ornamental plant: 625 kg/ha, broadcast throughout planting area or banding along the row of plant.
2. Fruit tree: 3-6 kg/plant, placement into the hole and cover with soil or application around the bush.
3. Seedling plot: 1-2 kg/10 m<sup>2</sup>, broadcast throughout planting area.



## 2.2 LDD 14 *Trichoderma harzianum*, wettable powder

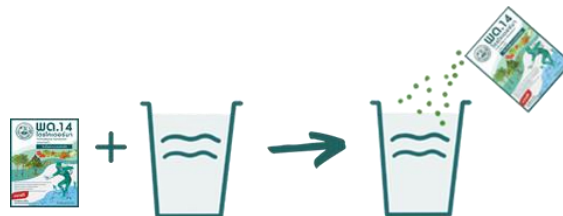


### How LDD14 works?

- LDD14 is a wettable powder formulation that forms a suspension when mixed with water and spray to foliage directly.
- Effective to control plant diseases of economic crops including, fruit trees, vegetables and rice.
- Life shelf is about 1 year.



### Utilization of LDD 14



- Mix 1 packs of LDD14 in 50 liters of water.
- Sprays at soil surface near tree base or directly foliage or trunk
- For field crops, vegetables and rice, sprays every 10 days continuously 2-3 times.
- For fruit tree and perennial plant, sprays to wound at stem or tree base every 10 days continuously 2-3 times until wound is relieve and dry.



## Benefits

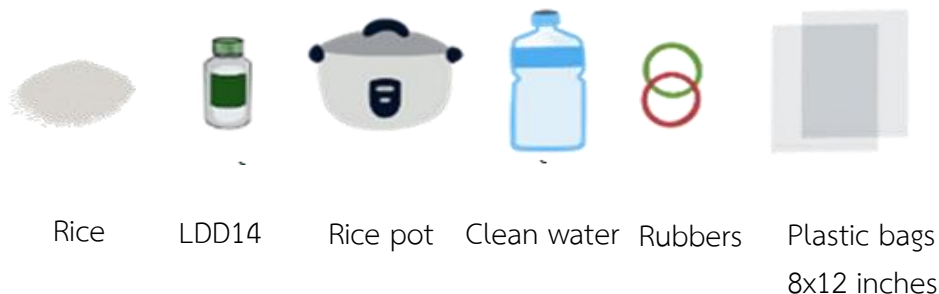
Inhibits various of plant diseases such as,

- Root and stem rot disease (*Phytophthora* spp.) in Durian, Rubber tree and Longan
- Leaf spot disease in rice (*Cercospora oryzae*)
- Leaf fall disease and anthracnose disease in chilli (*Colletotrichum gloeosporioides*)
- Leaf spot disease in vegetable (*Alternaria* spp.)
- Damping off disease in vegetable (*Pythium* spp. and *Sclerotium rolfsii*)
- Fusarium wilt (yellow) (*Fusarium oxysporum*)
- Bacterial soft rot disease (*Erwinia caratovora*) in vegetable
- Relieves root and stem rot symptoms
- Decreases using chemical pesticides



## Mass production of LDD 14

### Materials required



Rice

LDD14

Rice pot

Clean water

Rubbers

Plastic bags  
8x12 inches

### Inoculation method



1. Add 2 parts water and 1 part rice to a pot. Cook until the water is absorbed about 18 minutes

2. Put about 9 spoons of the rice into the plastic bags while the rice is still hot. Then fold the top of plastic bag to close and leave until it is cool.



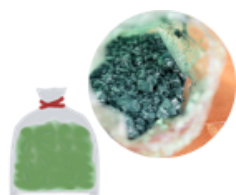




3. Pour 3-4 times LDD14 from the bottle to rice in plastic bag. Tie the plastic bag by rubber. Shake and mix together.



4. Pierce 10 holes the plastic bag by pin.



5. Trichoderma grow on rice completely about 4-5 days.



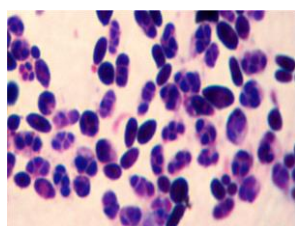
6. Fill 400 milliliters water into plastic bag. Rub rice with water until spores release all.



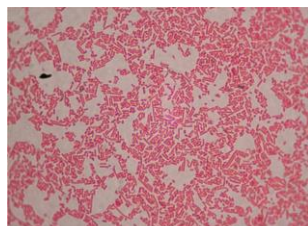
7. Mix 1,250 milliliters spores suspension and 32.25 liters of water prior spray in 1 ha agriculture area.

### 2.3 Microbial activator Super LDD 7

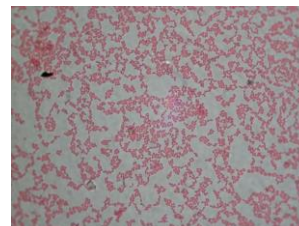
Microbial activator Super LDD 7 is a group of microorganisms which can active the process of fermentation and digestion of herbs for producing insect active ingredient. Super LDD 7 is composed of yeast (*Saccharomyces* sp.), acetic acid bacteria (*Gluconobacter oxydans*) and lactic acid bacteria (*Lactobacillus fermentum*)



*Saccharomyces* sp.



*Gluconobacter oxydans*








*Lactobacillus fermentum*








Biological extracts produced from fermented herbs by activity of microorganisms contains active ingredients, insect repellants and high amount of several kind of organic acids.

### Kind of herb for insect pest control

| Herb for insect pest control: mealybug and aphids                                 |   |   |   |   |
|---|---|---|---|---|
|  |  |  |  |  |
| Tobacco   | Long pepper   | Derris  | Asiatic bitter yam  | Chili   |

| Herb for insect pest control: common cutworm and short-hole worm                   |  |  |  |  |
|--|--|--|--|--|
|  |  |  |  |  |
| Sweet flag rhizomes  | Yam bean seed  | Neem seed  | Non Taai Yaak rhizomes   | Curcuma rhizomes   |

### Raw material to produce insect pest control substance by microbial activator Super LDD 7

#### Fermentation of fresh herb

|                                 |     |               |
|---------------------------------|-----|---------------|
| Herbs                           | 30  | kg            |
| Molasses or other sugar         | 10  | kg            |
| Rice bran                       | 100 | g             |
| Water                           | 30  | liters        |
| Microbial activator Super LDD 7 | 1   | package (25g) |

#### Fermentation of dry herb

|                                 |     |               |
|---------------------------------|-----|---------------|
| Herbs                           | 10  | kg            |
| Molasses or sugar               | 20  | kg            |
| Rice bran                       | 100 | g             |
| Water                           | 60  | liters        |
| Microbial activator Super LDD 7 | 1   | package (25g) |



## Step to produce insect pest control substance



1) Chop or pound each type of herbs into small pieces



4) Pour suspension into mixing tank and mix together.



2) Mix ground herb with rice bran thoroughly in mixing tank



5) Close a cover and put the tank under shady area, stir these substances every day, fermentation time 21 days.



3) Suspend Molasses or other sugar in water, pour microbial activator Super LDD 7, 5 minutes stirring.

### Consideration of completely fermentation process

- Reduction of microbial growth.
- Reduction of CO<sub>2</sub> bubble.
- Reduction of alcoholic smell.
- Dropping of sour smell.
- Dropping of pH down to 3-4.

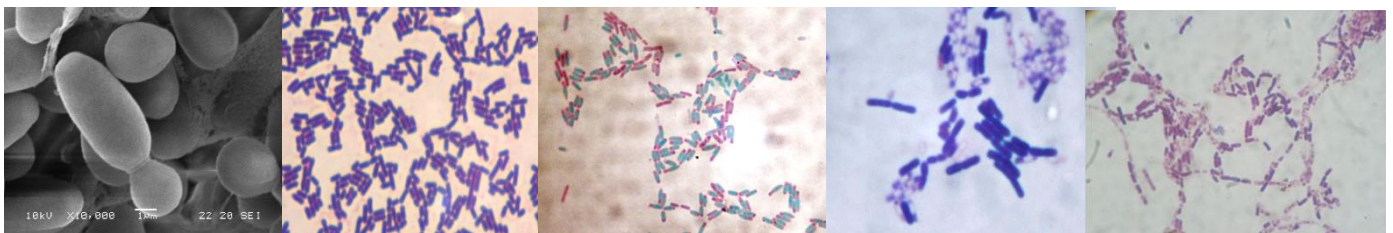
### Utilization and application rate of insect pest control from microbial activator Super LDD 7

- Dilute with water 1:100, spray every 3-5 days and spray continues 3 times in spread period of insect pest.
- Mix surfactant such as dishwasher 10 ml into insect pest control from microbial activator Super LDD 7 10 liters.
- Field crop Vegetable and flower: spray 313 liters in area 1 ha.
- Fruit tree: spray 625 liters in area 1 ha, leaf stem or insect.

### 3. Microbial for environment

Group of microbial for environment is microbial activator Super LDD 6 for waste water treatment, reduced odorous and controlling Culex mosquito larva population.

Microbial activator LDD 6 is a group of high efficient microorganisms which can activate fermentation and digestion process of kitchen waste such as residues from fruit, vegetable, fish and lipid from food and has bacteria control larva of urban mosquito for producing waste water treatment, reduced odorous, clean animal housing and control larva of Culex mosquito substance, which composed of yeast (*Saccharomyces cereviceae.*), lactic acid bacteria (*Lactobacillus fermentum*), proteolytic bacteria (*Bacillus cereus*), lipolytic bacteria (*Bacillus subtilis*) and mosquitocidal bacterial toxins (*Bacillus sphaericus*)



*Saccharomyces cereviceae*

*Lactobacillus fermentum*

*Bacillus cereus*

*Bacillus subtilis*

*Bacillus sphaericus*



## 1. Production waste water treatment and reduced odorous substance from kitchen wastes

### Raw material to produce waste water treatment and reduced odorous substance (50 liters)

|                                 |       |               |
|---------------------------------|-------|---------------|
| Kitchen wastes                  | 40    | kg            |
| Molasses                        | 10-20 | kg            |
| Water                           | 10    | liters        |
| Microbial activator Super LDD 6 | 1     | package (25g) |



### Step to produce waste water treatment and reduced odorous substance



1) Mix molasses or granulate sugar with water thoroughly in the mixing tank.



2) Suspend microbial activator Super LDD 6 1 pack in molasses or granulate sugar solution.



3) Stir continuously for 5 minutes.



4) Pour raw material into mixing tank and mix together.



5) Close a cover and put the tank under shady area, stir these substances every 2-3 days, fermentation time 20 days.

## 2. Production by cultivation of microbial activator Super LDD 6

|                                 |    |               |
|---------------------------------|----|---------------|
| Molasses                        | 5  | kg            |
| Water                           | 50 | liters        |
| Microbial activator Super LDD 6 | 1  | package (25g) |

### Procedure to cultivate



1. Mix molasses or granulate sugar with water thoroughly in the mixing tank.
2. Suspend microbial activator Super LDD 6 1 pack in molasses and stir continuously for 5 minutes.
3. Close a cover and put the tank under shady area, cultivation time 4 days and use rapidly.

### Utilization bacteria in microbial activator Super LDD 6 for controlling *Culex* Mosquito larva population

- *Culex* Mosquitos are vector for Japanese Encephalitis and Bancroftian Filariasis which habitat in waste water.
- *Bacillus sphaericus* produce specifically toxins with various mosquito types, mosquito larva. *B. sphaericus* are brought into gut of mosquito larva by eating. Base condition in the gut change crystal toxin of *B. sphaericus* from inactivated to activated form. The activated form toxins make gut wound, blood poisoning and finally the larva die.

### Utilization and application rate for waste water treatment and reduced odorous

- 1) Waste water treatment and use in Aquatic farms: use waste water treatment reduced odorous and control of urban mosquito larva substance 1 liter per water 10 m<sup>3</sup>, every 10 days.



2) Clean animal housing : dilute waste water treatment reduced odorous and control of urban mosquito larva substance with water 1 : 10, spray throughout treat area every day or every 3 days.



### Utilization and application rate for controlling urban mosquito larva population

Strew microbial activator Super LDD 6 1 package (25g) throughout treat area 100 m<sup>2</sup> and touch to urban mosquito larva.

