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Isolation and Identification of *Rhizopus oligosporus* Local Isolate Derived from Several Inoculum Sources

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ABSTRACT

This study was aims to obtain local isolates of *Rhizopus oligosporus* from several sources of inoculums such as Herbicus leaves, Tectona leaves, various brands of tempeh and starter of tempeh. *Rhizopus oligosporus* is a fungus that belongs to the *Zygomycetes* group, which is one of two classes in the Zygomycota phylum. Role of *R. oligosporus* in the popular Indonesian food Tempe fermentation. A total of 72 samples were isolated from various sources of inoculum consisting of 18 samples from waru leaves, 18 samples from teak leaves, 18 samples from various brands and the origin of tempe and 18 samples from various brands and origin of tempeh starter. The method used in this study was to isolate and identify *R. oligosporus* local isolates from waru leaves, teak leaves various brands of tempe and tempeh starter using Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA). The results showed that mold types *R. oryzae*, *R. oligosporus* and other molds with populations ranged from 1.5 x 10^2cfu /g - 9.5x 10^2cfu /g, 0.5 x 10^3 - 9.6 x 10^3cfu/g and 1.6 x 10^3 cfu /g-8.6 x 10^2cfu /g. A total of 72 samples taken from various sources of inoculums, *R. oligosporus* found as many as 12 from Herbicus leaves, 3 samples from Tectona leaves, 17 samples from various brands of tempeh and as many as 11 samples from tempeh starter were identified macroscopically based on color conidia with brownish gray color and the growth of mycelia and conidia is very dense. Macroscopically and microscopically identified as many as 19 local isolates of *R. oligosporus* which have the potential as selected fungi in food processing.

Introduction

R. oligosporus is a fungus of the family Mucoraceae and is a widely used starter culture for the production of tempeh at home and industrially. As the mold grows it produces fluffy, white mycelia, binding the beans together to create an edible "cake" of partly catabolized soybeans. The domestication of the microbe is thought to have occurred in Indonesia several centuries ago (Shurtleff and Aoyagi, 2001, A. 2001; Abe *et al.*, 2010; Jennessen *et al.*, 2008)

R. oligosporus is the preferred starter culture for tempeh production for several reasons. It grows effectively at high temperatures (30-40 °C) which are typical of the Indonesian islands, it exhibits strong lipolytic and proteolytic activity that create desirable properties in tempeh and it produces metabolites that allows it to inhibit and thus outcompete other molds and gram-positive bacteria, including the potentially harmful *Aspergillus flavus* and *Staphylococcus aureus* (Jennessen *et al.,* 2008; Hessel Tine *et al.,* 1940; Shurtleff and Aoyagi, 2001*). R. oligosporus* is at present considered to be a domesticated form of *Rhizopus microsporus* and its proper taxonomic position is thus *Rhizopus microsporus var. oligosporus*.

R. microsporus produces several potentially toxic metabolites, rhizoxin and rhizonins A and B, but it appears the domestication and mutation of the *R. oligosporus* genome has led to the loss of genetic material responsible for toxin production (Fardiaz 1989; Jennessen *et al.,* 2005; Madigan and Martinko, 2006)

Although other varieties in *Rhizopus microscopus* may be harmful, *R. oligosporus* is not associated with production of potentially harmful metabolites. It is not found in nature and is frequently used by humans (Pitt and Hocking,. 1985; Samson *et al.,* 2005; Dewi and Aziz, 2011) *Rhizopus oligosporus* strains have a large (up to 43 mm) and irregular spores with the most variable sizes.

This is, for instance, reflected as high values in the spore volume (96–223 mm3/spore). *R. oligosporus* has large, subglobose to globose spores, and high proportion irregular spores (>10 %). *R. oligosporus*also has spores with nonparallel valleys and ridges, and plateaus that sometimes are granular (Pitt and Hocking, 1985; Samson *et al.,* 2005). *R. oligosporus* Saito has brownish gray colonies with a height of 1 mm or more. Single sporangiofor or in groups with smooth or slightly rough walls, with a length of more than 1000 um and a diameter of 10-18 um. Sporangia globosa, which is brownish-black in color, with a diameter of 100-180 um (Pitt and Hocking, 1985; Samson *et al.,* 2005; Jennessen *et al.,* 2008). The research was aimed to isolation and identificatin of *R. oligosporus* derived from several leaf of plant and inoculums of tempeh and their starter. On the other hand *R. oligosporus* is used to inhibit *Aspergillus parasiticus* that is a patogen fungi harmfull of the heathy of human and animal. *Aspergillus parasiticus* was also produce secundary metabolite toxic aflatoxin B1.

Materials and Methods

This study was conducted from March 2018 to November 2018 in the Food Microbiology laboratory of the Food Science and Technology Study Program, Faculty of Agricultural Technology and Laboratory of Pest and disease of Plant, Faculty of Agriculture of Udayana University.

Sampling of Herbicus leaves, Tectona leaves, various brands and the origin of tempeh and various brands and origin of tempeh yeast in several provinces of Bali.

Rhizopus oligosporus Isolate

Local isolates of *R. oligosporus* in this study were obtained from isolation from various sources of inoculums, namely Herbicus leaves, Tectona leaves, various brands and origin of tempe and various brands and origin of tempeh starter in several provinces of Bali. Maintenance of isolates through regeneration every 3 months by inoculating on oblique Potato Dextrose agar (PDA) media and incubated 30oC for 7 days. Furthermore, it is stored in refrigerator 4o C.

Media isolation, purification, storage and maintenance of isolates

Media for growing molds using Potato Dextrose agar (PDA) agar media. Each 1000 ml medium was made from 200 g of potatoes, 20 g of glucose, 20 g of agar, 0.1 g of chloramphenicol and sterilized at 121o C for 15 minutes. The obtained fungal isolates were observed macroscopically and microscopically

Media identification (Pitt and Hocking, 1985)

Isolates on medium of Potato Dextrose agar (PDA) were then transferred to Malt Extract Agar (MEA) media, Czapek Yeast Extract Agar (CYA), 25% Glycerol Nitrate Agar (G25N) for identification. The composition of Malt Extract Agar (MEA) media per 1000 ml is 20 g of malt extract powder, 1.0 g of peptone, 20 g of glucose, 20 g of agar. The three media above were sterilized 121o C for 15 minutes. The composition of Czapek Yeast Extract Agar (CYA) per 1000 ml is 1.0 g K2HPO4, 10 ml czapek concentrate, 5.0 g yeast extract powder, 30 g sucrose, 15 g agar. While the composition of Czapek Concentrate in 100 ml is 30 g NaNO3, 5.0 g KCl, 5.0 g MgSO47H2O, 0.1 g FeSO4.7H2O. Media composition of 25% Glycerol Nitrate Agar (G25N) per 1000 ml is 0.75 g K2HPO4, 7.5 ml Czapek concentrate, 3.7 g yeast extract powder, 250 g glycerol, 12 g agar.

Isolation and purification of *Rhizopus* spp

The stages of isolation and purification of *Rhizopus* spp were carried out by taking aseptically the dominant isolate from tempeh which was inoculated on Potato Dextrose Agar (PDA) media and incubated at 30 ° C for 24 hours until a colony formed. Transfer of isolate (purification) is repeated 4 times until the isolate is completely pure. Flowchart of isolation and purification stages can be seen in Figure 1 (modified Pitt and Hocking, 1985)

Identification *R. oligosporus*

Mushroom isolates on oblique Potato Dextrose Agar (PDA) media are regrouped according to the origin of the inoculums. Identification was limited to isolates with brownish grayish conidia color using Czapek Yeast Extract Agar (CYA) media, 25% Glycerol Nitrate Agar (G25N), Malt Extract Agar (MEA) incubated for 7 days at 5 ° C, 25 ° C, and 37 ° C (modified Pitt and Hocking, 1985).

Results and Discussion

Population of *Rhizopus* spp.

The result of mold isolation from various sources of inoculums showed that from 72 samples studied found variations in mold types based on colony color and morphology both macroscopically and microscopically. In Table 1, the isolated fungi population from various sources of inoculums were shown.

R. oligosporus is a mold from the phylum Zygomycota which is able to produce protease enzymes. *R. oligosporus* is commonly found in rotting soil, fruits and vegetables, and old bread. *R. oligosporus* is included in Zygomycota which is often used in making tempeh from the fermentation process of soybeans, because *R. oligosporus* which produces phytase enzymes that break down phytate makes macro components in soybeans broken down into micro components so that tempeh is more easily digested and nutrients are more easily absorbed by the body. This fungus can also ferment other substrates, produce enzymes, and treat waste. One of the enzymes produced is from the protease group. (Rahayu, K. 1988; Abe *et al.,* 2010; Jennessen *et al.,* 2008)

R. oligosporus is a fungus of the family Mucoraceae and is a widely used starter culture for the production of tempeh at home and industrially. As the mold grows it produces fluffy, white mycelia, binding the beans together to create an edible "cake" of partly catabolized soybeans. The domestication of the microbe is thought to have occurred in Indonesia several centuries ago. (Hessel Tine *et al.*, 1940; Shurtleff and Aoyagi, 2001; Abe *et al.*, 2010; Jennessen *et al.*, 2006). In Table 2. Can be seen characteristic of *rhizopus spp* from *herbicus* leaves in macroscopically

R. oligosporus has brownish gray colonies with a height of 1 mm or more. A single person or group in groups with smooth or rather rough walls, with a length of more than 1000 micro meters and a diameter of 10-18 micro meters. Sporangia globosa is brownish black, with a diameter of 100-180 micro meters. Chlamydospores are many, single or short, colorless chains, containing granules, formed on hyphae, sporangiophores and sporangia. The form of Chlamidospora globosa, ellipse or cylindrical with a size of 7-30 micro meters or 12-45 micro meters x 7-35 micro meters (Pitt and Hocking, 1985; Samson *et al.*, 2005; Jennessen *et al.*, 2008)

R. oligosporus can grow optimally at a temperature of 30-35 ° C, with a minimum temperature of 12 ° C, and a maximum temperature of 42 ° C. Some of the benefits of *R. oligosporus* include its enzymatic activity, ability to produce natural antibiotics that specifically fight gram-positive bacteria, biosynthesis of vitamins B, its need for carbon and nitrogen source compounds, spore germination, and penetration of tempeh mycelia fungus into tissues soybean seeds (Kobayasi *et al.*,1992; Pitt and Hocking, 1985; Samson *et al.*, 2005; Jennessen *et al.*, 2008). In Table 3 can be seen characteristic of *rhizopus spp* from *tectona* leaves in macroscopically

R. oligosporus is the preferred starter culture for tempeh production for several reasons. It grows effectively at high temperatures (30-40°C) which are typical of the Indonesian islands, it exhibits strong lipolytic and proteolytic activity that create desirable properties in tempeh and it produces metabolites that allows it to inhibit and thus outcompete other molds and gram-positive bacteria, including the potentially harmful *Aspergillus flavus* and *Staphylococcus aureus* (Kobayasi *et al.*,1992; Rahayu, K. 1988; Shurtleff and Aoyagi, 2001; Madigan and Martinko, 2006). Table 4 can be seen characteristic of *rhizopus spp* from tempeh macroscopically *R. oligosporus* is at present considered to be a domesticated form of *R. microsporus* and its proper taxonomic position is thus *R. microsporus var. oligosporus*. *R. microsporus* produces several potentially toxic metabolites, rhizoxin and rhizonins A and B, but it appears the domestication and mutation of the *R. oligosporus* genome has led to the loss of genetic material responsible for toxin production (Jennessen *et al.*, 2006; Samson *et al.*, 2005; Jennessen *et al.*, 2008). Table 5. Can be seen characteristics of *rhizopus spp* from starter of tempeh macroscopically

Macroscopically and microscopically isolates of *Rhizopus* spp with brownish gray color are presented in Figures 1, 2 and 3.

To support species determination or to evaluate the possibilities of adopting infraspecific classification of Zheng *et al.*, (2007) for those 35 strains in *R. microsporus*, morphological and physiological characteristics were then observed.

Morphological characteristics, such as sporangiophore (length and colour), columellae (shape), sporangiospore (shape, size, and colour), and rhizoidetype were examined according to Zheng *et al.*, (2007) by using light microscope Olympus™BX53 (Olympus, Japan). Measurements of sporangiophore length and sporangiospore size were made in 30 replications (n ¼ 30).

Table.1 Population of molds from several of inoculums

Inoculom sources	*R. oryzae* (Cfu/g)	*R. oligosporus* (Cfu/g)	Other molds (Cfu/g)
Herbicus leaves 1	5.0×10^2	6.3×10^3	1.7×10^2
Herbicus leaves 2	5.2×10^2	8.2×10^3	2.8×10^2
Herbicus leaves 3	5.6×10^2	9.1×10^3	1.6×10^2
Tectona leaves 1	8.4×10^2	0.5×10^2	8.6×10^2
Tectona leaves 2	7.6×10^2	1.0×10^2	7.9×10^2
Tectona leaves 3	9.5×10^2	1.2×10^2	6.6×10^2
Tempeh 1	0	7.2×10^3	0
Tempeh 2	0	9.6×10^3	0
Tempeh 3	0	$8{,}0 \times 10^3$	0
Starter of Tempeh 1	2.5×10^2	$9{,}5 \times 10^3$	0
Starter of Tempeh 2	2.2×10^2	$6{,}5 \times 10^3$	0
Starter of Tempeh	1.5×10^2	$4{,}5 \times 10^3$	0

Table.2 Characteristic of *Rhizopus spp* from *Herbicus* leaves in macroscopically

No	Code of Isolate	Sources of Inoculums	Color of Colony	Observation in medium agar	
				Mycelia	Conidia
1	WS1	Sanur	Brownish Gray	+++	+++
2	WS2	Sanur	Brownish Gray	+++	+++
3	WS3	Sanur	Brownish Gray	+++	+++
4	WR1	Renon	Brownish Gray	+++	+++
5	WR2	Renon	Brownish Gray	+++	+++
6	WR3	Renon	Brownish Gray	+++	+++
7	WP1	Padanggalak	gray	++	++
8	WP2	Padanggalak	gray	++	++
9	WP3	Padanggalak	gray	++	++
10	WK1	Kesiman	Brownish Gray	+++	+++
11	WK2	Kesiman	Brownish Gray	+++	+++
12	WK3	Kesiman	Brownish Gray	+++	+++
13	WJ1	Jimbaran	gray	+++	+++
14	WJ2	Jimbaran	gray	++	+++
15	WJ2	Jimbaran	gray	++	+++
16	WB1	Bukit	Brownish Gray	+++	+++
17	WB2	Bukit	Brownish Gray	+++	++
18	WB3	Bukit	Brownish Gray	+++	++

Remarks : +++ : Very Thick (cover all over PDA sloped), ++ : Thick (Cover ¾ PDA sloped on 5 days)

Table.3 Characteristic of *Rhizopus spp* from *Tectona* leaves in macroscopically

S.No.	Code Isolate	Sources of Inoculums	Color of Colony	Observation in medium agar	
				Miselia	konidia
1	JJ1	Jimbaran	Gray	+++	++
2	JJ2	Jimbaran	Gray	+++	++
3	JJ3	Jimbaran	Gray	++	++
4	JF1	Kampus FTP	Gray	+++	+++
5	JF2	Kampus FTP	Gray	+++	+++
6	JF3	Kampus FTP	Gray	++	++
7	JT1	Kampus Teknik	Brownish Gray	+++	+++
8	JT2	Kampus Teknik	Brownish Gray	+++	+++
9	JT3	Kampus Teknik	Gray	+++	++
10	JM1	Kampus MIPA	Gray	+++	+++
11	JM1	Kampus MIPA	Gray	+++	+++
12	JM1	Kampus MIPA	Gray	++	++
13	JS1	Singaraja	Brownish Gray	++	+++
14	JS2	Sigaraja	Gray	++	++
15	JS3	Singaraja	Gray	+++	+++
16	JK1	Denpasar	Gray	++	+++
17	JK2	Denpasar	Gray	++	++
18	JK3	Denpasar	Gray	+++	+++

Remarks : +++ : Very Thick (cover all over PDA sloped), ++ : Thick (Cover ¾ PDA sloped on 5 days)

Table.4 Characteristic of *Rhizopus spp* from Tempeh Macroscopically

No	Code Isolate	Sources of Inoculums	Color of Conidia	Observation in Medium Agar	
				Miselia	konidia
1	TT1	None Merk	Brownish Gray	+++	+++
2	TT2	None Merk	Brownish Gray	+++	+++
3	TT3	None Merk	Brownish Gray	+++	+++
4	TL1	Merk Langgeng	Brownish Gray	+++	+++
5	TL2	Merk Langgeng	Brownish Gray	+++	+++
6	TA1	Merk Arya	Brownish Gray	+++	+++
7	TA2	Merk Arya	Brownish Gray	+++	+++
8	TF1	Merk Family	Brownish Gray	+++	+++
9	TF2	Merk Family	Brownish Gray	+++	+++
10	TM1	Merk Murni	Brownish Gray	+++	+++
11	TM2	Merk Murni	Brownish Gray	+++	+++
12	TP1	None Merk	Brownish Gray	+++	+++
13	TP2	None Merk	Brownish Gray	+++	+++
14	TC1	Merk Cak	Brownish Gray	+++	+++
15	TC2	Merk Cak	Brownish Gray	+++	+++
16	TG1	None Merk	Brownish Gray	+++	+++
17	TG2	None Merk	Brownish Gray	+++	+++
18	TS1	None Merk (Singaraja)	Brownish Gray	+++	+++

Remarks : +++ : Very Thick (cover all over PDA sloped), ++ : Thick (Cover ¾ PDA sloped on 5 days)

Tabe.5 Characteristics of *Rhizopus spp* from Starter of Tempeh Macroscopically

No	**Code Isolate**	**Sources of Inoculums**	**Color of Conidia**	**Observation in Mediaum Agar**	
				Miselia	konidia
1	RP1	Ragi Reprima	Gray	+++	+++
2	RP2	Ragi Reprima	Gray	+++	+++
3	RG1	Ragi Pemogan	Gray	+++	+++
4	RG2	Ragi Pemogan	Brownish Gray	+++	+++
5	RI 1	Ragi perusahan di Seririt	Brownish Gray	+++	+++
6	RI 2	Ragi perusahan di Seririt	Brownish Gray	+++	+++
7	RA1	Ragi Perusahan di Pulau Saelus	Gray	+++	+++
8	RA2	Ragi Perusahan di Pulau Saelus	Brownish Gray	+++	+++
9	RR1	Ragi tanpa Merk (Singaraja)	Brownish Gray	+++	+++
10	RR2	Ragi tanpa Merk (Singaraja)	Brownish Gray	+++	+++
11	RL1	Padangsambian	Brownish Gray	+++	+++
12	RL2	Padangsambian	Brownish Gray	+++	+++
13	RT1	Ragi tanpa merk (Sesetan)	Gray	+++	+++
14	RT2	Ragi tanpa merk (sesetan)	Brownish Gray	+++	+++
15	RF1	Ragi tanpa merk (Ubung)	Gray	+++	+++
16	RF2	Ragi tanpa merk (Ubung)	Brownish Gray	+++	+++
17	RC2	Ragi tanpa merk (Sesetan)	Brownish Gray	+++	+++
18	RC3	Ragi tanpa merk (Sesetan)	Gray	+++	+++

Remarks : +++ : Very Thick (cover all over PDA sloped), ++ : Thick (Cover ¾ PDA sloped on 5 days)

Tabel.6 Observation macroscopic and Microscopes 10 isolate *R.oligosporus* from several inoculums

Profile	**Code Isolate**										***R. oligosporus***
	WR	JT	TT	TL	TA	TF	TM	TP	TC	TG	
Color mycelia	white	white	white	white	white	white	white	White	white	white	White*
Color Conidia	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray
Shape Conidia	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-ellipsoidal (Oval)
Length Conidia	11.4	14.8	10	12	12	14.8	11.4	11.4	10	10	7-24 µm
Length Sporangiosphore	160	150-160	160.5	170	170	170	170	160	170	170	150-400 µm
Length sporangium	90	90	90	100	82.5	100	100.5	100.4	80	80	80-120 µm
Texture Sporangiosphore	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Length Columela	27.1	27	25	25	30	30	25	25	27	27	25 -27 µm *
Shape Columela	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	sub globose-Globose
Chlamidosphore	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Abundant, single or Short Chain **

* Samson et al., 2005; ** Jennessen et al., 2008

Tabel.7 Observation macroscopic and Microscopes 10 isolate *R.oligosporus* from several inoculums

Profile	**Code Isolate**									***R. oligosporus***
	TS	RG	RI	RA	RR	RL	RT	RF	RC	
Color mycelia	white	white	white	white	white	white	white	white	white	White*
Color konidia	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray	Brownish Gray
Shape Conidia	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Globose-Elipsoidal (Oval)	Elipsoidal (Oval)	Elipsoidal (Oval)	Elipsoidal (Oval)	Globose	Elipsoidal (Oval)	Elipsoidal (Oval)	Globose-ellipsoidal (Oval)
Length Conidia	10	10	12	12	12	20	12	20	12	7-24 µm
Length Sporangiosphore	170	150	170	170	170	170.1	170.1	170	170	150-400 µm
Length sporangium	80	80	90	90	90	90.1	92	85	90	80-120 µm
Tekstur Sporangiospore	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Length kolumela	27	27	27.1	27.1	27.1	27.1	27.1	27.1	27.1	25 -27 µm *
Bentuk Columela	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	Globose-sub globose	sub globose-Globose
Clamidosphore	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Single-Short Chain	Abundant, single or Short Chain **

* Samson et al., 2005; ** Jennessen et al., 2008

Fig.1 Color of colony and mycelia *Rhizopus* spp from Herbicus leaves, Tectona leaves, Tempeh and starter of tempeh Incubation 3 days temetarure 30°C

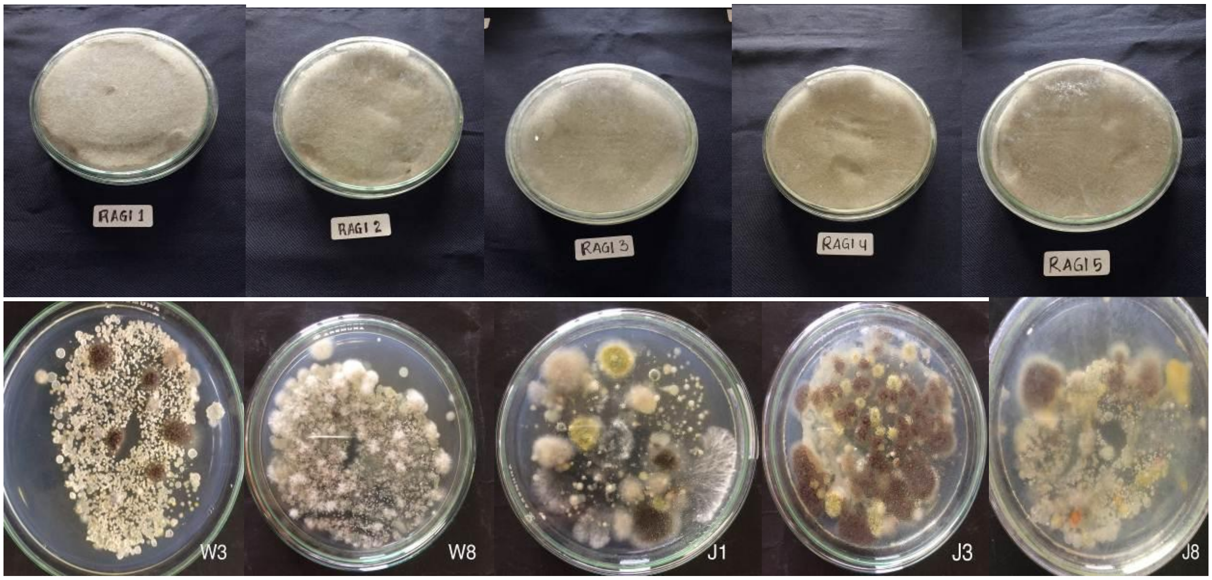


Fig.2 Purification Isolate *R. oligosporus* Incubation 5 days in macroscopically

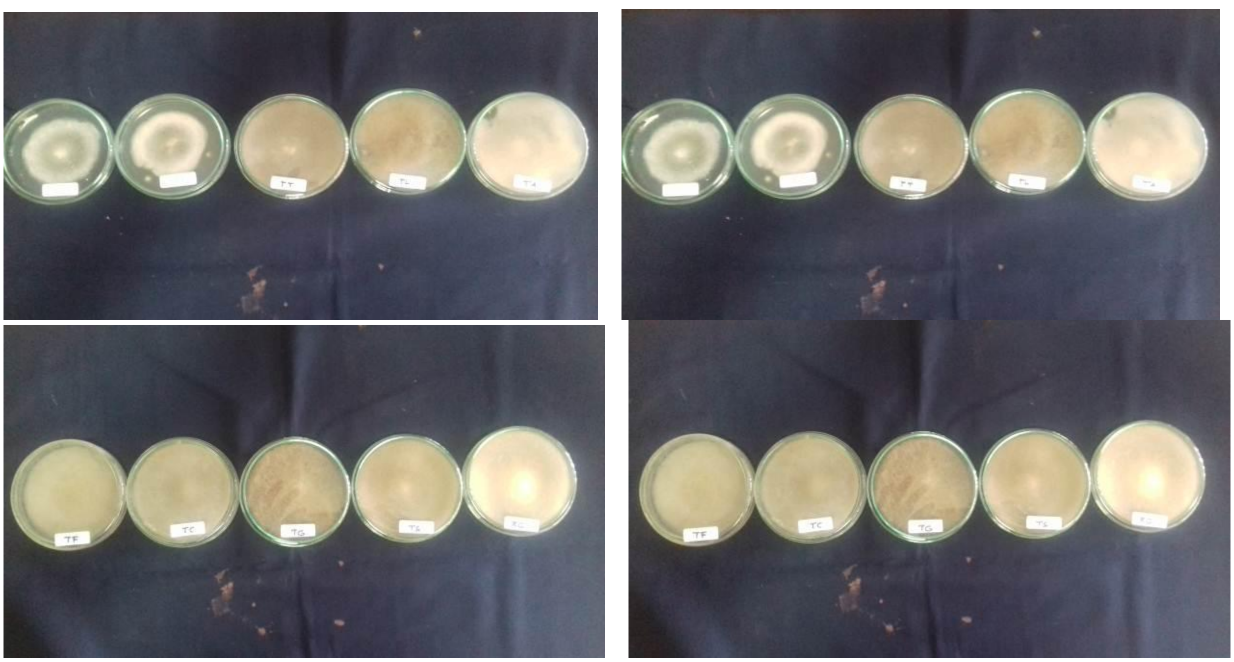
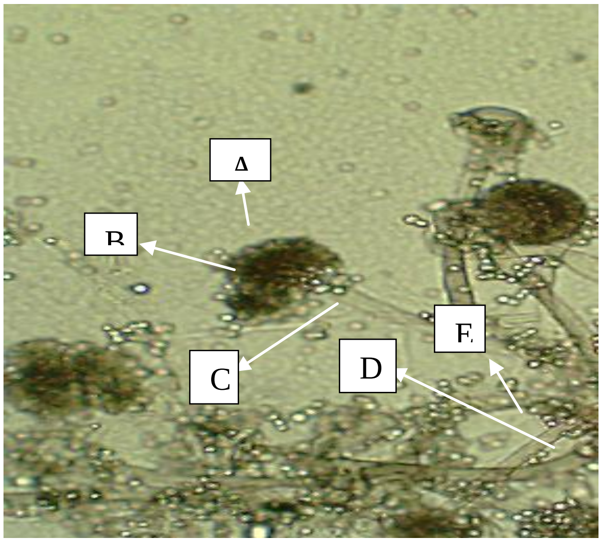
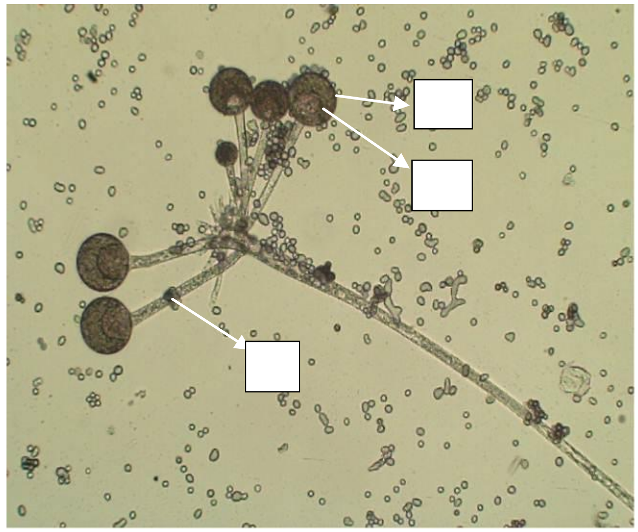


Fig.3 Morphology *Rhizopus oligosporus* (400 x) (A=Conidia, B= Sporangiosphore, C= Conidiophore, D= Stolon dan E = Rhizoid

Isolate from Herbicus Leaves **Isolate from Tectona leaves**





Isolate from Tempeh **Isolate from Starter of Tempeh**

In physiological characterization, the ability of Rhizopus spp. to grow at 33^{o}C, 42^{o}C, 46^{o}C, 48^o C, and 51^{o}C was examined.

The group of fungi that has the most role in making tempe is the genus Rhizopus. Rhizopus sp molds has been known for a long time as a fungus that plays a major role in the process of fermentation of soybeans into tempeh. Rhizopus sp will form a compact white solid called a fine thread / biomass.

Fine yarn / biomass is caused by the fungus mycelia growing on the surface of the soybean seeds and connecting the soybean seeds. Rhizopus sp types are so diverse that they need to be isolated and their morphology and characteristics identified. Identification based on fungal morphology is by observing sporangiophores, sporangium and sporangio-spores (Jennessen *et al.*, 2008; Dewi and Aziz 2011; Chang-Tien *et al.*, 2009)). *R. oligosporus* is a fungus that belongs to the class Zygomycetes, which is one of two classes in the phylum Zygomycota.[5] *R. oligosporus* belongs to the *Rhizopus microsporus* group. This group is made of taxa with similar morphology that are associated with undesired metabolite production, pathogenesis and food fermentation. Although other varieties in *R. microscopus* may be harmful, *R. oligosporus* is not associated with production of potentially harmful metabolites. It is not found in nature and is frequently used by humans.[6] *R. oligosporus* strains have a large (up to 43 mm) and irregular spores with the most variable sizes. This is, for instance, reflected as high values in the spore volume (96–223 mm3/spore).*R. oligosporus* has large, subglobose to globose spores, and high proportion irregular spores (>10 %). *R. oligosporus*also has spores with nonparallel valleys and ridges, and plateaus that sometimes are granular (Yanai *et al.*, 1992; Hessel Tine, 1940; Pitt and Hocking, 1985; Samson *et al.*, 2005)

R. oligosporus can grow optimally at a temperature of 30-35 ° C, with a minimum temperature of 12 ° C, and a maximum temperature of 42 ° C. Growth of R. oligosporus has the characteristics of brownish gray colonies with a height of 1 mm or more. Single sporangiophores or in groups with smooth or slightly rough walls, with a length of more than 1000 µm and a diameter of 10-18 µm. Sporangia globosa which when brown is black brown in color, with a diameter of 100-180 µm. Chlamydospores are many, single or short, colorless chains, containing granules, formed on hyphae, sporangiophores and sporangia. The form of klamidospora globosa, ellipse or cylindrical with a size of 7-30 µm or 12-45 µm x 7-35 µm (Madigan and Martinko, 2006; Pitt and Hocking, 1985; Samson *et al.,* 2005). Table 6 can be seen 10 isolate *R.oligosporus* from several inoculums

Indonesia in this study belonging to *R. delemar* and *R. microsporus*. Indeed, several members of Rhizopus, such as *R. oligosporus*, *R. oryzae*, *R. arrhizus,* and *R. stolonifer* were previously reported in Indonesia from inoculants of tempeh (ragi) and from fresh tempeh (Dewi and Aziz 2011; Prihatna and Suwanto 2007). In the current systematic of Rhizopus, *R. arrhizus* is treated as a synonym of *R. oryzae* a s proposed by Abe *et al.,* (2010)

A popular Indonesian food, Tempeh, is created by fermenting soybeans in combination with *R. oligosporus* (Dewi and Aziz 2011; Chang-Tien *et al.,* 2009; Prihatna and Suwanto 2007) In order to create tempeh, soybeans must first be soaked in water (usually overnight) at a temperature similar to the environment it is placed in. The soybean's outer covering is then removed and the beans are partially cooked. Lactic acid bacteria, like Lactococcus and Lb. casei species, play a major role in the fermentation of tempeh.[8] For the tempeh to ferment there needs to be a suitable, pure inoculums. Also, spores with a tendency for fast germ inability are needed, as well (Caplice, *et al.,* 1999; Prihatna and Suwanto 2007). In order for the tempeh to attain its characteristic compact 'cake' form after fermentation, the soybeans become compressed due to the mycelia of *R. oligosporus*.[8] Rapidly growing mycelia helps speed up the growth of this fungus. Because mycelia are quite sensitive to dehydration and adverse temperatures, preserving tempeh for extended periods of time can be challenging (Dewi and Aziz 2011; Chang-Tien *et al.,* 2009; Prihatna and Suwanto 2007) When the soybeans are bound together by the white mycelium, the fungus releases enzymes that make this heavily protein-rich product more digestible for humans (Tempeh-like foods can also be created from cereal grains such as wheat and rice. Many times, a good inoculum for this new fermentation actually comes from tiny pieces of old tempeh that have already been fermented (Caplice, *et al.,* 1999; Prihatna and Suwanto 2007; Chang-Tien *et al.,* 2009)

Isolates other than 72 isolates that were successfully isolated (besides the 19 isolates) there were 24 isolates including the genus Rhyzopus (data not supported). All isolates were characterized by nonseptic hyphae, had stolons and rhizoids that were black when was old, sporangiophora growing basically also contained rhizoid, large and black sporangia, larger columella and apophysis, such as jackets, buying sporangia at the time of sporangiofora, growing, and also containing rhizoid, large and black sporangia, larger columella and apophysis, such as a jacket, buying sporangia at the time of sporangiofora, increasing, and forming mycelium like cotton as argued by Fardiaz (1989; Dewi and Aziz (2011); Chang-Tien *et al.,* (2009); Prihatna and Suwanto (2007)). Table 7. Can be seen 9 isolate *R.oligosporus* from several inoculums *R. oryzae, R. oligosporus* and other mold types were found, with populations ranging from 1.5 x 102cfu / g - 9.5x 102 cfu / g, 0.5 x 103 - 9.6x

103cfu / g and 1.6 x102cfu / g-8.6 x 102cfu / g A total of 72 samples taken from various sources of inoculums, *R. oligosporus* found as many as 12 from waru leaves, 3 samples from teak leaves, 17 samples from various brands of tempeh and as many as 11 samples from tempe yeast were identified macroscopically based on color conidia with brownish gray color and the growth of mycelia and conidia is very dense. Macroscopically and microscopically identified as many as 19 local isolates of *R. oligosporus* which have the potential as selected fungi in food processing.

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References

Abe A, Oda Y, Asano K, Sone T. 2010. A molecular phylogeny based taxonomy of the genus Rhizopus. Biosc Biotechn Biochem 74:1325e31. http://dx.doi.org/10.1271/bbb.90718.

Caplice, Elizabeth; Fitzgerald, Gerald F. (1999). "Food Fermentations: role of microorganisms in food production and preservation". International Journal of Food Microbiology. 50: 143. doi:10.1016/S0168-1605(99) 00082-3. Retrieved 27 May 2014

Chang-Tien, Chang; Hsu, Cheng-Kuang; Chou, Su-Tze; Chen, Ya-Chen; Huang, Feng-Sheng; Chung, Yun-Chin (2009). "Effect of fermentation time on the antioxidant activities of tempeh prepared from fermented soybean using Rhizopus oligosporous". International Journal of Food Science and Technology. 44: 799. doi:10.1111/j.1365-2621.2009.01907.x. Retrieved 27 May 2014

Dewi RS and Aziz S. 2011. Isolasi Rhizopus oligosporus pada beberapa inokulum tempe di Kabupaten Banyumas. Molekul 6:93e104.

Fardiaz S., 1989, *Mikrobiologi Pangan*, PAU-IPB, Bogor

Hessel Tine, W; Swain, E.W.; Wang, Hwa L. (1940). "Mass production of Rhizopus oligosporus spores and their application in tempeh fermentation" (PDF). Journal of Food Science. 40: 168–170. doi:10.1111/j.1365-2621.1975.tb03762.x. Retrieved 29 May 2014.

Jennessen, J.; Nielsen, K.F.; Houbraken, J.; Lyhne, E.K.; Schnürer, J.; Frisvad, J.C.; Samson, R.A. (2005). "Secondary metabolite and mycotoxin production by the Rhizopus microsporus group". Journal of Agricultural and Food Chemistry. 53: 1833–1840. doi:10.1021/jf048147n. PMID 15740082.

Jennessen, J., J. Schnurer, J. Olsson, R.A. Samson, and J. Dijksterhuis, 2008, Morphological characteristics of sporangiospores of the tempe fungus Rhizopus oligosporus differentiate it from other taxa of the R. microsporus group. Mycol. Res, Vol.112, 547-63.

Kobayasi, Sin-ya; Naoto, OKAZAKI; Takuya, KosEKI (1992). "Purification and Characterization of an Antibiotic Substance Produced from Rhizopus oligosporus IFO 8631". Biosci. Biotechnol. Biochem. 56 (1): 94–98. doi:10.1271/bbb.56.94. PMID 1368137.

Madigan, M.T., and J.M. Martinko, 2006, Brock Biology of Microorganisms 11th ed. Pearson Education, New Jersey.

Pitt, J.I. and A.D Hocking,. 1985. Fungi and Food Spoilage. Academic Press, Australia.

Prihatna C, Suwanto A. 2007. Phenotypic, metabolic, and genetic diversity of the Indonesian strains of Rhizopus oligosporous. J Microbiol Indones 1:27-32

Rahayu, K. 1988. Bahan Pengajaran Mikrobiologi Pangan PAU Pangan dan Gizi.UGM, Yogyakarta.

Samson, R.A., E.S. Hoekstra, J.C. Frisvad and O. Filtenborg. 1995. Introduction to Food-Borne Fungi. Baarn and Lyngby, Netherlands

Shurtleff, W. & Aoyagi, A. 2001. The book of tempeh. 2 2. Ten Speed Press. Berkeley, California pp.

Nout, M.J.R. (1989). "Effect of Rhizopus and Neurospora spp. on growth of Aspergillus flavus and A. parasiticus and accumulation of aflatoxin B1 in groundnut". Mycological Research. 93: 518–523. doi:10.1016/s0953-7562(89)80046-2.

Yanai, K; Takaya, N; Kojima, N; Horiuchi, H; Ohta, A; Takagi, M (1992). "Purification of two chitinases from Rhizopus oligosporus and isolation and sequencing of the encoding genes". American Society for Microbiology: 7398. Retrieved 29 May 2014.

Zheng RY, Chen GQ, Huang H, Liu XY. 2007. A monograph of rhizopus. Sydowia 59:273-372

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