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

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**A B S T R A C T**

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 10² cfu/g - 9.5 x 10² cfu/g, 0.5 x 10³ - 9.6 x 10³ cfu/g and 1.6 x 10³ cfu/g-8.6 x 10³ cfu/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.

**Keywords**
Identification, *R. oligosporus*, local isolate, Inoculums.

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**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 microsporus* 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 plateaux 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 identification 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 pathogen fungi harmful of the health of human and animal. *Aspergillus parasiticus* was also produce secondary 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 Hericicus 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 Hericicus 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 4oC.

**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 121° 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 121° 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 MgSO4.7H2O, 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 *hericus* 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 Martinho, 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), columnellae (shape), sporangiospore (shape, size, and colour), and rhizoidtype 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 = 30).

1088
Table 1: Population of molds from several inoculums

| Inoculum sources | R. oryzae (Cfu/g) | R. oligosporus (Cfu/g) | Other molds (Cfu/g) |
|------------------|------------------|------------------------|-------------------|
| Herbicus leaves 1| 5.0 x 10²         | 6.3 x 10³              | 1.7 x 10²         |
| Herbicus leaves 2| 5.2 x 10²         | 8.2 x 10³              | 2.8 x 10²         |
| Herbicus leaves 3| 5.6 x 10²         | 9.1 x 10³              | 1.6 x 10²         |
| Tectona leaves 1 | 8.4 x 10²         | 0.5 x 10²              | 8.6 x 10²         |
| Tectona leaves 2 | 7.6 x 10²         | 1.0 x 10²              | 7.9 x 10²         |
| Tectona leaves 3 | 9.5 x 10²         | 1.2 x 10²              | 6.6 x 10²         |
| Tempeh 1          | 0                | 7.2 x 10³              | 0                 |
| Tempeh 2          | 0                | 9.6 x 10³              | 0                 |
| Tempeh 3          | 0                | 8.0 x 10³              | 0                 |
| Starter of Tempeh 1| 2.5 x 10²      | 9.5 x 10³              | 0                 |
| Starter of Tempeh 2| 2.2 x 10²      | 6.5 x 10³              | 0                 |
| Starter of Tempeh | 1.5 x 10²         | 4.5 x 10³              | 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)
### Table 5: Characteristics of *Rhizopus spp* from Starter of Tempeh Macroscopically

| No | Code Isolate | Sources of Inoculums                | Color of Conidia | Observation in Medium Agar |
|----|--------------|------------------------------------|------------------|----------------------------|
| 1  | RP1          | Ragi Reprimax                       | Gray             | +++                        |
| 2  | RP2          | Ragi Reprimax                       | Gray             | +++                        |
| 3  | RG1          | Ragi Pemogan                        | Gray             | +++                        |
| 4  | RG2          | Ragi Pemogan                        | Brownish Gray    | +++                        |
| 5  | RI 1         | Ragi perusahaan di Seririt          | Brownish Gray    | +++                        |
| 6  | RI 2         | Ragi perusahaan di Seririt          | Brownish Gray    | +++                        |
| 7  | RA1          | Ragi Perusahaan di Pulau Saelus     | Gray             | +++                        |
| 8  | RA2          | Ragi Perusahaan 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* |
|------------------------------|--------------|------------------|
| **Color mycelia**            | WR           | white            |
|                              | JT           | white            |
|                              | TT           | white            |
|                              | TL           | white            |
|                              | TA           | white            |
|                              | TF           | white            |
|                              | TM           | White            |
|                              | TP           | White            |
|                              | TC           | white            |
|                              | TG           | 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| Brownish Gray    |
|                              | Brownish Gray| Brownish Gray    |
|                              | Brownish Gray| Brownish Gray    |
| **Shape Conidia**            | Globose-Elipsoidal (Oval)| Globose-Elipsoidal (Oval) |
|                              | Globose-Elipsoidal (Oval)| Globose-Elipsoidal (Oval) |
|                              | Elipsoidal (Oval)     | Elipsoidal (Oval) |
|                              | Elipsoidal (Oval)     | Elipsoidal (Oval) |
|                              | Elipsoidal (Oval)     | Elipsoidal (Oval) |
|                              | Elipsoidal (Oval)     | Elipsoidal (Oval) |
|                              | Elipsoidal (Oval)     | Elipsoidal (Oval) |
| **Length Conidia**           | 11.4          | 11.4             |
|                              | 14.8          | 14.8             |
|                              | 10            | 10               |
|                              | 12            | 12               |
|                              | 12            | 12               |
|                              | 14.8          | 14.8             |
| **Length Sporangiosphore**   | 160           | 160.5            |
|                              | 150-160       | 170              |
|                              | 160.5         | 170              |
|                              | 170           | 170              |
|                              | 170           | 170              |
|                              | 160           | 170              |
|                              | 170           | 170              |
| **Length Sporangium**        | 90            | 90               |
|                              | 90            | 90               |
|                              | 100           | 82.5             |
|                              | 100           | 100              |
|                              | 100.5         | 100.4            |
|                              | 80            | 80               |
| **Texture Sporangiosphore**  | Smooth        | Smooth           |
|                              | Smooth        | Smooth           |
|                              | Smooth        | Smooth           |
|                              | Smooth        | Smooth           |
|                              | Smooth        | Smooth           |
|                              | Smooth        | Smooth           |
|                              | Smooth        | Smooth           |
| **Length Columela**          | 27.1          | 27               |
|                              | 27            | 30               |
|                              | 25            | 30               |
|                              | 25            | 25               |
|                              | 25            | 25               |
|                              | 27            | 27               |
| **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 |
|                              | Globose-sub globose| Globose-sub globose |
|                              | Globose-sub globose| Globose-sub globose |
|                              | Globose-sub globose| Globose-sub globose |
| **Chlamidosphere**           | 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 |
|                              | 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
Table 7 Observation macroscopic and microscopes 10 isolate *R. oligosporus* from several inoculums

| Profile                  | Code Isolate | \(R. \text{oligosporus}\) |
|--------------------------|--------------|----------------------------|
| **Color mycelia**        |              |                            |
| TS                       | white        | white                      |
| RG                       | white        | white                      |
| RI                       | white        | white                      |
| RA                       | white        | white                      |
| RR                       | white        | white                      |
| RL                       | white        | white                      |
| RT                       | white        | white                      |
| RF                       | white        | White*                     |
| RC                       | white        | White*                     |
| **Color konidia**        |              |                            |
| TS                       | Brownish Gray| Brownish Gray              |
| RG                       | Brownish Gray| Brownish Gray              |
| RI                       | Brownish Gray| Brownish Gray              |
| RA                       | Brownish Gray| Brownish Gray              |
| RR                       | Brownish Gray| Brownish Gray              |
| RL                       | Brownish Gray| Brownish Gray              |
| RT                       | Brownish Gray| Brownish Gray              |
| RF                       | Brownish Gray| Brownish Gray              |
| RC                       | Brownish Gray| Brownish Gray              |
| **Shape Conidia**        |              |                            |
| TS                       | Globose-Elipsoidal (Oval) | Globose-ellipsoidal (Oval) |
| RG                       | Globose-Elipsoidal (Oval) | Globose-ellipsoidal (Oval) |
| RI                       | Globose-Elipsoidal (Oval) | Globose-ellipsoidal (Oval) |
| RA                       | Elipsoidal (Oval) | Elipsoidal (Oval)         |
| RR                       | Elipsoidal (Oval) | Elipsoidal (Oval)         |
| RL                       | Elipsoidal (Oval) | Elipsoidal (Oval)         |
| RT                       | Elipsoidal (Oval) | Elipsoidal (Oval)         |
| RF                       | Elipsoidal (Oval) | Elipsoidal (Oval)         |
| RC                       | Elipsoidal (Oval) | Elipsoidal (Oval)         |
| **Length Conidia**       |              | 7-24 μm                    |
| TS                       | 10           | 12                         |
| RG                       | 10           | 12                         |
| RI                       | 12           | 12                         |
| RA                       | 12           | 20                         |
| RR                       | 20           | 12                         |
| RL                       | 20           | 12                         |
| RT                       | 12           | 12                         |
| RF                       | 12           | 7-24 μm                    |
| RC                       | 12           | 150-400 μm                 |
| **Length Sporangiosphere** | 170     | 170.1                      |
| TS                       | 170          | 170                        |
| RG                       | 170          | 170                        |
| RI                       | 170.1        | 170.1                      |
| RA                       | 170          | 170                        |
| RR                       | 170.1        | 170.1                      |
| RL                       | 170          | 170                        |
| RT                       | 170          | 170                        |
| RF                       | 170          | 170                        |
| RC                       | 170          | 150-400 μm                 |
| **Length sporangium**    |              | 80-120 μm                  |
| TS                       | 80           | 85                         |
| RG                       | 80           | 85                         |
| RI                       | 90           | 85                         |
| RA                       | 90           | 85                         |
| RR                       | 90.1         | 85                         |
| RL                       | 92           | 85                         |
| RT                       | 85           | 85                         |
| RF                       | 90           | 80-120 μm                  |
| RC                       | 90           | 80-120 μm                  |
| **Tekstur Sporangiospore** | Smooth     | Smooth                     |
| TS                       | Smooth       | Smooth                     |
| RG                       | Smooth       | Smooth                     |
| RI                       | Smooth       | Smooth                     |
| RA                       | Smooth       | Smooth                     |
| RR                       | Smooth       | Smooth                     |
| RL                       | Smooth       | Smooth                     |
| RT                       | Smooth       | Smooth                     |
| RF                       | Smooth       | Smooth                     |
| RC                       | Smooth       | Smooth                     |
| **Length kolumela**      |              | 25 -27 μm *                |
| TS                       | 27           | 27                         |
| RG                       | 27           | 27                         |
| RI                       | 27.1         | 27.1                       |
| RA                       | 27.1         | 27.1                       |
| RR                       | 27.1         | 27.1                       |
| RL                       | 27.1         | 27.1                       |
| RT                       | 27.1         | 27.1                       |
| RF                       | 27.1         | 27.1                       |
| RC                       | 27.1         | 27.1                       |
| **Bentuk Columela**      |              | sub globose-Globose        |
| TS                       | Globose-sub globose | Globose-sub globose |
| RG                       | Globose-sub globose | Globose-sub globose |
| RI                       | Globose-sub globose | Globose-sub globose |
| RA                       | Globose-sub globose | Globose-sub globose |
| RR                       | Globose-sub globose | Globose-sub globose |
| RL                       | Globose-sub globose | Globose-sub globose |
| RT                       | Globose-sub globose | Globose-sub globose |
| RF                       | Globose-sub globose | Globose-sub globose |
| RC                       | Globose-sub globose | Globose-sub globose |
| **Clamidosphore**        |              | Abundant, single or Short Chain ** |
| TS                       | Single-Short Chain | Single-Short Chain        |
| RG                       | Single-Short Chain | Single-Short Chain        |
| RI                       | Single-Short Chain | Single-Short Chain        |
| RA                       | Single-Short Chain | Single-Short Chain        |
| RR                       | Single-Short Chain | Single-Short Chain        |
| RL                       | Single-Short Chain | Single-Short Chain        |
| RT                       | Single-Short Chain | Single-Short Chain        |
| RF                       | Single-Short Chain | Single-Short Chain        |
| RC                       | Single-Short Chain | Single-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 temperature 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°C, 42°C, 46°C, 48°C, and 51°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 sporangiospores (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. microsporus* 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)
*Rhizopus 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* as 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. 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*. 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 x 102 cfu / g - 8.6 x 102 cfu / 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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