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**Submission date:** 15-Aug-2018 06:49PM (UTC+0700)

**Submission ID:** 990134603

**File name:** JAST- \_ARTIKEL\_ -2014.pdf (680.44K)

**Word count:** 2604

**Character count:** 13143

# Preparation of Dried Culture of Probiotic *Rhizopus oryzae* in Two Different Mediums as Feed Additive to Indigenous Chicken

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Received: September 5, 2014 / Published: October 20, 2014.

**Abstract:** Fungus *Rhizopus oryzae* has a probiotic potential, but the potency is not yet explored. Probiotics are usually used as feed additive, including to the chicken. Probiotic needs a suitable medium for their life. The aim of the study was to know which preparations can produce suitable medium for the growth of *Rhizopus oryzae* on the dried culture formed. The study used two preparations. Preparation 1 (a) consisted of rice bran (1 kg) and culture of *Rhizopus oryzae* on Potato Dextrose Broth (PDB medium) (500 mL); then preparation 1 (b) consisted of corn grain (1 kg) and culture of *Rhizopus oryzae* on PDB medium (500 mL). Preparation 2 (a) consisted of rice bran (1 kg), culture of *Rhizopus oryzae* on Potato Dextrose Agar (PDA medium) (seven petri dishes) and was added with sterile distilled water (500 mL); preparation 2 (b) consisted of corn grain (1 kg), culture of *Rhizopus oryzae* on PDA medium (seven petri dishes) and was added with sterile distilled water (500 mL). The culture of *Rhizopus oryzae* even both on PDB and PDA were 3 d of incubation. All preparations were incubated for 7 d then they were dried under the sun. Parameter evaluation was the growth performance of the fungus in the dried culture media. The growth performance was analyzed by microscopic analysis and the number of colony of the fungi in the two dried culture media. The results showed that from the macroscopic analysis, the sample of both dried culture media which were placed on PDA medium showed the growth of mycelia and spores of the same kind of fungus and it was proved by microscopic observation that the fruiting bodies (mycelia and spores) of that fungus was *Rhizopus oryzae*. The colonies number of the fungi from preparation 1 (a) and 1 (b) were  $0.4 \times 10^3$  cfu/gr and  $2.4 \times 10^3$  cfu/gr, respectively, and from preparation 2 (a) and 2 (b) were  $1.3 \times 10^5$  cfu/gr and  $8.3 \times 10^2$  cfu/gr, respectively. It could be concluded that the product of the dried culture of probiotic *Rizopus oryzae* from the preparation 2 with rice brand or corn grain mediums can be used as feed additive to indigenous chicken.

**Key words:** Feed additive, probiotic, dried culture, chicken.

## 1. Introduction

Probiotic is alive microorganism that can be used as feed additives [1, 2]. Probiotics will succeed on their functions if their amounts are sufficient and still alive when they reach in the intestine of the host [3-5]. The probiotic can improve the microbial balance of the digestive tract, improve the development of the small intestine and contribute to the growth of the host [5-8].

Microorganisms which are well known as probiotics mostly belong to bacteria [7]. In fact, the microorganisms which belong to probiotic are not only bacteria. Whereas, some fungi also have that potency but have not even been explored yet. For example, one fungus has the potency as a probiotic, i.e., *Rhizopus oryzae* [5]. Like most other fungi, fungi which have probiotic potencies for their growing also need a suitable conditions such as pH, water capacity, temperature and substrate or medium [1, 9, 10]. These conditions are also needed when probiotics are produced as feed additive product.

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As feed additive product, probiotic is usually packed in liquid form. Whereas, the packaging is very risky in any conditions. The alternative form which is more safety than the other forms are dried culture. Making the probiotic's dried culture needs an appropriate substrate or medium. The medium especially for the probiotic that was used as feed additive should also be liked by the host animal.

In this study, it produced probiotic's dried culture and the product will be used as feed supplement to indigenous chicken. The medium or substrate for the growth of the probiotic was rice bran and corn grain. Both media belong to the material feeds that are liked by chicken. Additionally, the products are prepared in two preparation ways.

The objective of this study was to know which preparation can produce suitable medium for the growth of *Rhizopus oryzae* on dried culture. The best preparation will be applied in producing these probiotic and the product will be fed to indigenous chicken.

## 2. Materials and Methods

The study was done on Maret–Mei 2014 in Laboratory of Physiology and Biochemistry in Animal and Agricultural Science Faculty, and Laboratory of Microbiology in Faculty of Science and Mathematic, Diponegoro University, Semarang, Central Java, Indonesia.

Materials used in this study were rice bran, corn grain, culture isolate of *Rhizopus oryzae* on Potato Dextrose Broth (PDB) and Potato Dextrose Agar (PDA) mediums (3 d incubation), sterile distilled water. In this study, there were two preparations. Preparation 1 (a) was rice bran (1 kg) and culture isolate of *Rhizopus oryzae* on PDB medium (500 mL); preparation 1 (b) was corn grain (1 kg) and culture isolate of *Rhizopus oryzae* on PDB medium (500 mL). Preparation 2 (a) was rice bran (1 kg), culture isolate of *Rhizopus oryzae* on PDA medium (seven petri

dishes) and was added with sterile distilled water (500 mL); preparation 2 (b) was corn grain (1 kg), culture isolate of *Rhizopus oryzae* on PDA medium (seven petri dishes) and was added with sterile distilled water (500 mL). All preparations were replicated three times.

Firstly, the cultures of the isolate *Rhizopus oryzae* is done by regrowing it on a new PDA in petri dish and on PDB medium in erlenmeyer flask. Then it was incubated in temperature 37 °C. The aged *Rhizopus oryzae* isolate both on PDB and PDA mediums were 3 d of incubation. Rice bran and corn grain were placed in plastic bags and sterilized in autoclave before they were inoculated with the isolates. All the prepared media above were incubated for 7 d. In addition, the prepared media were shaken thoroughly every twice days. After incubation, then they were dried.

Parameter evaluation was the growth performance of the fungus *Rhizopus oryzae* in the dried culture media. Analysis used macroscopic, microscopic performances and the total colonies number of fungi in the two samples of both dried culture media. Macroscopic analysis was done by regrowing the samples of all dried cultures media on PDA for 2 d. After they grew, then the isolate was also used for microscopic analysis. The microscopic analysis was done by taking the fruiting bodies (mycelia and spores) of the fungus from the macroscopic preparation above and then being observed under the microscope. Accounting the total number of colony of fungi used dilutions plate methods.

## 3. Results

### 3.1 The Product of Probiotic Culture of *Rhizopus oryzae* in Two Different Media

The preparations of the products are two forms of probiotic cultures—wet form (Fig. 1) and dried form (Fig. 2). The cultures are grown in two mediums, i.e., rice bran and corn grain.

Preparation of Dried Culture of Probiotic *Rhizopus oryzae* in Two Different Mediums as Feed Additive to Indigenous Chicken

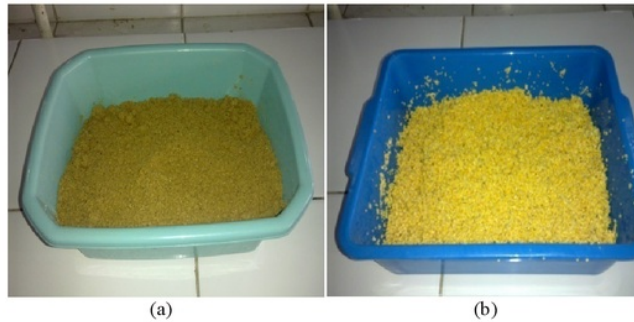


Fig. 1 Wet culture form: (a) rice bran medium, (b) corn grain medium.

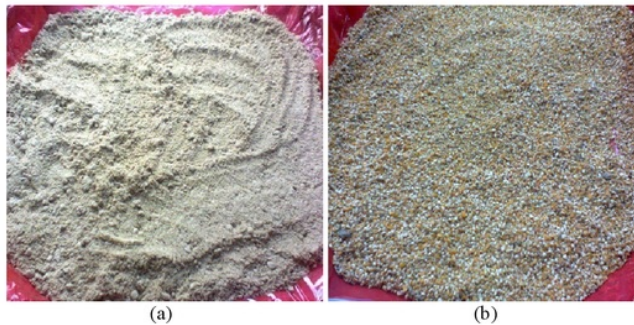


Fig. 2 Dried culture form: (a) rice brand medium, (b) corn grain medium.

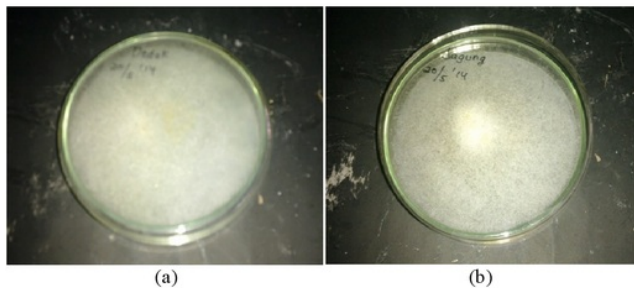


Fig. 3 Fungi growth on PDA medium of the preparation 1: (a) rice brand, (b) corn grain.

### 3.2 Macroscopic Performances of Probiotic Dried Culture Product of *Rhizopus oryzae*

The results of macroscopic evaluation of the products in the dried culture form were showed in Figs. 3 and 4. Macroscopic performances of the products were showed by the growth profusely of mycelia and spores of certain fungi which covered on the surfaces of the PDA medium in the petri dish. The colour of mycelia of the fungi was white and after 2 d incubation, in the tip of mycelia, spores with brownish

colour appeared. The kind of fungi was not yet known and this will be identified in the microscopic evaluation.

### 3.3 Microscopic Performances of Probiotic Dried Culture Product of *Rhizopus oryzae*

The results of microscopic evaluation of dried culture products in two different mediums were showed in Figs. 5a and 5b. The microscopic performances were got from the mycelia and spores

which were taken from the fungi (Figs. 3 and 4), then were observed under the microscope and the result was species *Rhizopus oryzae*.

### 3.4 The Colonies Number of Fungi in Probiotic Dried Culture Products

The results of the colonies number of fungi in the two probiotic dried cultures which were prepared in two ways were shown in Table 1. The data showed that the colonies number of fungi which are got from preparation 2 was bigger than from preparation 1. The colonies number of rice bran medium from the preparation 2 was the biggest one, then followed by corn grain from the preparation 1 and corn grain from preparation 2, and the fewest was rice bran medium from the preparation 1.

## 4. Discussion

The results of the macroscopic performance showed that mycelia and spores of the same kind of fungus grew profusely on PDA in petri dishes (Figs. 3 and 4). The characteristic of the fungus were white colour and after 2 d later the centre tip of mycelia changed the colour from white to become brownish. This result indicated that in the dried culture forms of both, i.e., the same fungi grew in the rice bran and corn grain media. The identification by the microscopic evaluation showed that the fungi was *Rhizopus oryzae* [11]. The character of fungi was that the mycelia is whitish becoming brownish grey. These findings mean that in the probiotic dried culture products in this study, there are profusely fruiting bodies of the fungus *Rhizopus*

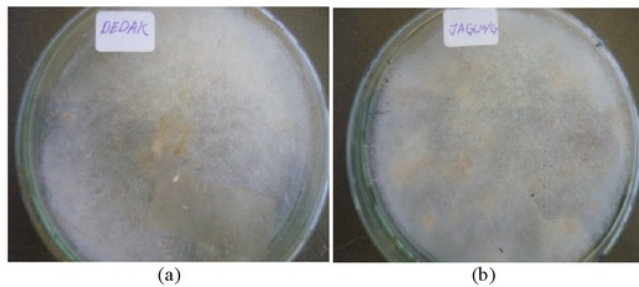


Fig. 4 Fungi growth on PDA medium of the preparation 2: (a) rice bran, (b) corn grain.

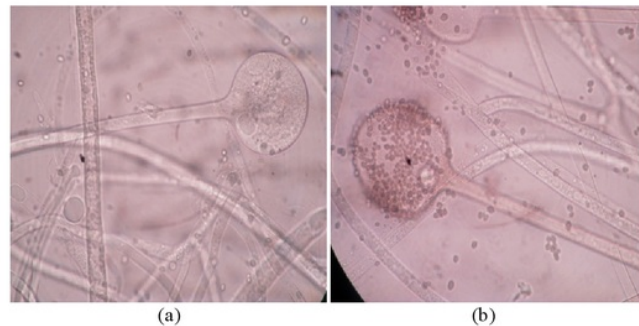


Fig. 5 Mycelia and spores of *Rhizopus oryzae* (400x): (a) rice bran, (b) corn grain.

Table 1 The colonies number of fungi in probiotic dried culture products.

Colonies number of fungi (cfu/gr)	Preparation 1		Preparation 2		Mixed all dried culture media
	Rice bran	Corn grain	Rice bran	Corn grain	
	$0.4 \times 10^1$	$2.4 \times 10^3$	$1.3 \times 10^5$	$8.3 \times 10^2$	$1.0 \times 10^5$

*oryzae* both in the rice bran and corn grain (Figs. 3-5). The products also showed that rice bran and corn grain could also be suitable mediums for growing fungus. The same results also found in Ref. [5] that a fungus can grow in rice bran and corn grain, even though the fungus was not *Rhizopus oryzae* but *Chrysonilia crassa*.

*Rhizopus oryzae* belongs to the group of *Rhizopus* sp. which easily grew in all conditions. As mention in Ref. [1] that the fungus belong to the fungi and they can grow easily in any condition and any media. Because of their character, it makes the fungi grow easily in any conditions and any media, such as in the dried culture form in this study.

PDA and PDB mediums used in this study belong to the common media [9]. Those fungi grow easily in common medium [9, 12]. The both chose media in this study was right because it was proved by the result that the isolate of *Rhizopus* sp. grew profusely in that media.

The data from Table 1 showed that the colonies number of fungi which are got from preparation 2 was bigger than that from preparation 1. In general, the medium which are used in this study, i.e., rice brand and corn grain can grew the fungi. These findings showed that the preparations with those mediums could be used for making dried culture of fungus, especially for *Rhizopus oryzae*.

The results showed that there are differences of the colonies number of fungi which were got from different preparations (Table 1). The differences could be caused by the adding of sterile distilled water into the medium. In preparation 1, it was not added with any water, but in preparation 2 it was added with 500 mL of sterile distilled water. Thus, the media in the preparation 2 has better moisture condition than that in the preparation 1. This condition can contribute to the growing of the fungi. The effect showed that the colonies number of the fungi in both media in the preparation 2 got more larger number than preparation 1 (Table 1). These findings are as same as that in Ref.

[9] statement that the moisture is the important factor for growing fungi and the low level of fungi like *Rhizopus oryzae* needs a moisture about 90%.

## 5. Conclusions

The conclusion was that the preparation 2 with rice brand or corn grain mediums are the best preparation to produce probiotic dried culture and the product of the dried culture of probiotic *Rizopus oryzae* from the preparation 2 with rice brand or corn grain mediums can be used as feed additive to indigenous chicken.

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