

# In Vitro Test of Probiotic Strain of Seven Species of Fungi Which Isolated from Gastrointestinal Tract of Indigenous Chicken

*by* Turrini Yudiarti

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Saeri Sagiman  
Sutarman Gafur  
Zakiatulyaqin

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## Preface

As the Organizing Committee of The 6 International Seminar of Indonesian Society for Microbiology, 2013, in Aston Hotel Pontianak, we proudly present this Seminar Proceeding to the participants and to the society members. This proceeding is an important parts of dissemination process of experimental results from diverse expertise in Indonesian Society for Microbiology. Dissemination of experimetal results need to be done continuously so that the process of knowledge transfer could be accelerated.

The topic of the seminar is: Unlimited use of Microbia. There were 114 papers presented and 38 posters, however, there is only 32 of selected papers to be published in this proceeding. We greatly appreciate to all the participants who contributing for the seminar to be a great success. This seminar is an annual routine agenda of Indonesian Society for Microbiology. The objective were to disscus the current issues on microbiology, to innitiate knowledge transfer, and to trigger research collaboration and publications among the society members.

We, the Organizing Committee, deeply appologize if there is any mistake or any inconvenient we have made not only during seminar activities but also in this proceeding publication. We hope this proceeding will be very useful for you all. Finally, special thank for the auditors who have made this proceeding successfully published.

Dr. Ir. Sutarman Gafur, M.Sc.  
Chairman of Organizing Committee

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# **In Vitro Test of Probiotic Strain of Seven Species of Fungi Which Isolated from Gastrointestinal Tract of Indigenous Chicken**

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## **Abstract**

Probiotic is a live microbial feed supplement which beneficially affects the host animal. Microbe which belong to probiotic must have some characters such as tolerance to gastric condition, bile salt and have antimicrobial activity against potential pathogen of gastrointestinal tract. The objective of the research was in vitro test of probiotic strain of seven species of fungi that have been isolated from gastrointestinal tract of the indigenous chicken. The species were *Aspergillus fumigatus*, *Aspergillus niger*, *Chrysonilia crassa*, *Mucor circinelloides*, *Mucor sp.*, *Rhizopus oligosporus*, and *Rhizopus oryzae*. The test used two methods and two media. Parameter observed was the growth of the seven species of fungi in the gastric conditions (pH 3 & 8), bile salt solution with two concentrations (0.4 & 0.8%), and their growth together with two potential pathogens i.e. *Aspergillus flavus* and *Eschericia coli* in the same medium. Results showed that species which belong to the most potential to less potential as a probiotic strain were as follows *Chrysonilia crassa*, *Mucor circinelloides*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Mucor sp.*, *Aspergillus niger*, and *Aspergillus fumigatus*.

*Key words* : fungi, probiotic, in vitro test

## **I. Introduction**

The term probiotic is currently used when it refers to microbe associated with beneficial effects on humans and animals (Corcionivoschi et al., 2010). Some ways are needed to select and characterise the probiotic strains. The criteria of probiotic strain such as tolerance to conditions of the digestive tract, antibacterial factors, and safety to the host.

Probiotic is not only bacteria, but other microbe like fungi can also be as a probiotic. Fungi are belong to microorganism which have a definite cell wall, they are usually nonmotile, although they may have more reproductive cells and they reproduce spore (Alexopoulos and Mims, 1979). They can live in the gastrointestinal tract of chicken (Kompiang et al., 2006; Lumpkins et al., 2010, Yudiarti et al., 2012).

Fungi that have been known as a probiotic e.g. *Saccharomyces cerevisiae*, *Aspergillus niger* and *Aspergillus oryzae* (Koenen et al., 2002, Lee et al., 2006, Mountzouris et al., 2007, Lee and Salminen, 2009). We previously have found seven species of fungi which were isolated from the gastrointestinal tract of the indigenous chicken (Yudiarti et al., 2012a). Those species were *Aspergillus fumigatus*, *Aspergillus niger*, *Chrysonilia crassa*, *Mucor circinelloides*, *Mucor sp.*, *Rhizopus oligosporus*, and *Rhizopus oryzae*. Whereas those fungi were not tested for their potency as a probiotic strain yet.

The objective of this research was to select and characterise for probiotic strain of the seven species of those fungi by the in vitro test..

## II. Research Methods

The research was done on June – October 2011 in the Laboratory of Physiology and Biochemistry in Animal and Agricultural Science Faculty, Diponegoro University. Firstly, It was made renewed of all cultures by regrowing them on a new potato dextrose agar (PDA) medium. Then incubated in the temperature 40°C for 48 hours.

The procedure of the first method was : after the isolates profusely grew on PDA then it was took 2 cm diameters of the medium of each culture and placed on treatment medium. The treatment media were acid medium (pH 3), base medium (pH 8) and media adds with bile salt concentration 0.4 and 0.8 %. Then all plates were incubated in the temperature 40°C for 48 hours. All treatments were replicated three times.

The second method was : one plate of each isolates of each fungus was deep and shaken in to the treatment solutions about 10 to 15 minutes in the erlenmeyer glass. The treatment solutions were solution with pH 3, pH 8, and bile salt solution with concentration 0.4 and 0.8%. Then it was pipetted of 1 ml of each treatment solution and placed on PDA neutral medium. All treatment plates were incubated in the temperature 40°C for 48 hours. All treatments were replicated three times.

The procedure of test of growing together with the pathogen of gastrointestinal tract (*Aspergillus flavus* or *Eschericia coli*) was : each medium on plate was placed one side with fungus as candidate of probiotic and the other side with one isolate of potential pathogen. All treatments were incubated in the temperature 40°C for 48 hours and replicated three times. Thereafter it was measured for growing of all species in all conditions.

## III. Results and Discussion

### The growth of seven species of fungi in acid (pH 3) and base conditions (pH8)

The growth of seven species of fungi on acid (pH 3) and base condition (pH 8) with first and second methods shown in Table 1 and 2. Statistical analysis showed there was an effect of acid and base condition to the growth of seven species of fungi. Duncan test showed that there were significant difference ( $P<0,05$ ) between all treatments (Table 1 and 2).

Table 1. The Growth Rates of Seven Species of Fungi on Acid Condition (pH 3)

Species Fungi	First Method -----cm-----	Second Method -----%-----
<i>Aspergillus fumigatus</i>	0,27 <sup>f</sup>	55,00 <sup>d</sup>
<i>Aspergillus niger</i>	0,50 <sup>f</sup>	30,00 <sup>e</sup>
<i>Chrysonilia crassa</i>	3,77 <sup>a</sup>	100,00 <sup>a</sup>
<i>Mucor circinelloides</i>	3,23 <sup>b</sup>	99,17 <sup>a</sup>
<i>Mucor sp</i>	0,82 <sup>c</sup>	90,00 <sup>b</sup>
<i>Rhizopus oligosporus</i>	1,45 <sup>d</sup>	92,50 <sup>b</sup>
<i>Rhizopus oryzae</i>	1,85 <sup>c</sup>	82,50 <sup>c</sup>

Different superscripts within the same column indicate significantly different ( $P<0,05$ )

Table 2. The Growth Rates of Seven Species of Fungi on Base Condition (pH 8)

Species of Fungi	First Method -----cm-----	Second Method -----%-----
<i>Aspergillus fumigatus</i>	0,27 <sup>bc</sup>	49,17 <sup>d</sup>

<i>Aspergillus niger</i>	0,30 <sup>b</sup>	23,33 <sup>e</sup>
<i>Chrysonilia crassa</i>	1,73 <sup>a</sup>	100,00 <sup>a</sup>
<i>Mucor circinelloides</i>	0,20 <sup>bcd</sup>	90,00 <sup>c</sup>
<i>Mucor sp</i>	0,15 <sup>cd</sup>	100,00 <sup>a</sup>
<i>Rhizopus oligosporus</i>	0,22 <sup>bed</sup>	95,00 <sup>b</sup>
<i>Rhizopus oryzae</i>	0,47 <sup>d e</sup>	95,00 <sup>b</sup>

Different superscripts within the same column indicate significantly different (P<0,05)

Table 1 and 2 shown that the growth of *Chrysonilia crassa* on acid (pH 3) and base condition (pH 8) with first and second methods tend to has better adaptability than others species. Those because the fungus is belong to the fast growing fungus. This was showed by the research of Yudiarti (2012a) that the growth of colony of *Chrysonilia crassa* on PDA at 40°C in 24 hours attained a diameter 4 – 4.5 cm, but others fungi were *Aspergillus fumigatus* 0.8 -1.0 cm, *Aspergillus niger* 0.2 – 0.5 cm, *Mucor circinelloides* 4 – 4.5 cm, *Mucor sp* 2.0 - 2.3 cm, *Rhizopus oligosporus* 2.6 – 3.5 cm and *Rhizopus oryzae* 0.7 - 1 cm. Lindeberg et al. (1982) also contributed that *Chrysonilia crassa* could grow on pH 1.5 – 8.0. Because of the potency, *Chrysonilia crassa* could easily adapted in all conditions.

## 2 The growth of seven species of fungi in bile salt solution

2 The growth of all species of fungi in bile salt solution in two concentrations (0.4 and 0.8%) shown in Table 3. Statistical analysis showed there was an effect of bile salt solution with different concentrations to the growth of seven species of fungi. Duncan test showed that there were significant difference (P<0,05) between all treatments (Table 3).

Table 3. The Growth of Seven Species of Fungi on Bile Salt Solution

Species of Fungi	Bile Salt Concentrations	
	0,4%	0,8%
	----- % -----	
<i>Aspergillus fumigatus</i>	12,50 <sup>e</sup>	11,67 <sup>c</sup>
<i>Aspergillus niger</i>	65,83 <sup>c</sup>	13,33 <sup>c</sup>
<i>Chrysonilia crassa</i>	71,67 <sup>b</sup>	67,50 <sup>b</sup>
<i>Mucor circinelloides</i>	87,50 <sup>a</sup>	85,00 <sup>a</sup>
<i>Mucor sp</i>	60,83 <sup>d</sup>	70,83 <sup>b</sup>
<i>Rhizopus oligosporus</i>	90,83 <sup>a</sup>	85,00 <sup>a</sup>
<i>Rhizopus oryzae</i>	46,33 <sup>a</sup>	82,50 <sup>a</sup>

Different superscripts within the same column indicate significantly different (P<0,05)

Table 3 shown that all species could growth in two different concentrations of bile salt solution even some species were not grow well. Whereas three species that were *Mucor circinelloides*, *Rhizopus oligosporus*, and *Rhizopus oryzae* were more tolerance than other fungi.

The results shown that two species which belong to genus *Rhizopus* i.e *Rhizopus oligosporus* and *Rhizopus oryzae* could grow better than others. It may be because the genus could produce exocellular lipase which is very similar to pancreatic lipase. In gastrointestinal tract those enzyme is always interaction with bile salt to degrade lipid (Noma and Borgstrom, 1971). As same as the genus *Rhizopus*, the genus *Mucor* also could produce that enzyme (Ogiso and Sugiura, 1969). Owing to that potency, when the fungi were growth in bile salt solution they were easily adapted.

2

## The Growth of Seven Species of Fungi with Two Pathogen on the Medium

2

The growth of seven species of fungi with two pathogens (*Aspergillus flavus* and *Escherichia coli*) in the same medium shown in Table 4. The most species of fungi in this research could growth more than the pathogens. Two species i.e *Chrysonilia crassa* and *Mucor circinelloides* showed that in the surrounding them there were free space between the two fungi and the pathogens. This indicated that the fungi secreted substances that could inhibit the growth of pathogens.

Table 4. The Growth of Seven Species of Fungi with Two Pathogens on Medium.

Species of Fungi	Species of Pathogen	
	<i>Aspergillus flavus</i> (AF <sub>L</sub> )	<i>Escherichia coli</i> (EC)
<i>Aspergillus fumigatus</i> (AF)	AF < AF <sub>L</sub>	AF > EC, AF covered EC
<i>Aspergillus niger</i> (AN)	AN > AF <sub>L</sub>	AN > EC, AN covered EC
<i>Chrysonilia crassa</i> (CC)	CC > AF <sub>L</sub> There was a free space between them	CC > EC CC covered EC
<i>Mucor circinelloides</i> (MC)	MC > AF <sub>L</sub> There was a free space between them	MC > EC There was a free space between them
<i>Mucor spesies</i> (MS)	MS > AF <sub>L</sub>	MS > EC
<i>Rhizopus oligosporus</i> (RO <sub>L</sub> )	RO <sub>L</sub> > AF <sub>L</sub>	RO <sub>L</sub> > EC, CC covered EC
<i>Rhizopus oryzae</i> (RO <sub>R</sub> )	RO <sub>R</sub> > AF <sub>L</sub>	RO <sub>R</sub> = EC

Note : > = growth more than  
< = growth less than

Result of the research (Table 4) shown that all species of fungi could be an antagonist to the pathogens of gastrointestinal tract. This potency it may be contributed by the potencies of the fungi like strong cell wall which is resistance to enzyme attacked. Beside that the fungi could hydrolyze toxic to be a simple substance which can be used for them or other microorganism (Ganjar et al., 2006). So with the potencies the fungus when competes with pathogen it could easily to be a winner. Research by Yudiarti (2012 b) also showed that the fungi e.g. *Chrysonilia crassa* was used as feed additive of the chicken shown that the fungus could decreased the number of the bacteria and fungi in the gastrointestinal tract of the chicken. It might be the decreasing number was the number of pathogens.

1

## Overall In Vitro Test of Potency of Probiotic Strain of Seven Species of Fungi

1

Result of overall test of potency of probiotic strain of seven species of fungi shown in Tabel 5. The species of the fungi which belong to the most potential to less potential as a probiotic strain were *Chrysonilia crassa*, *Mucor circinelloides*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Mucor sp*, *Aspergillus niger*, and *Aspergillus fumigatus*. From all the tests, one species i.e. *Chrysonilia crassa* was more potential than others. Those are showed by the result that six of eight tests, the fungus could grow 100 %. It means that the fungus could grew and covered the surface of medium (Tabel 5).

Tabel 5. Overall Test of In vitro of Potency of Probiotic Strain of Seven Species of Fungi

Spesiec of Fungi	Acid (pH3)		Base (pH8)		Bile salt concentration		Growth with Two pathogens	
	Padat	Cair	Padat	Cair	0,4%	0,8%	AF	E.Coli
<i>Aspergillus fumigatus</i>	+	+	+	+	+	+	+	+++
<i>Aspergillus niger</i>	+	+	++	+	+	+	+++	+++
<i>Chrysonilia crassa</i>	+++	+++	+++	+++	++	++	+++	+++
<i>Mucor circinelloides</i>	++	+++	+	+	+++	+++	+++	+++
<i>Mucor sp</i>	+	++	+	+++	+	++	+++	+++
<i>Rhizopus oligosporus</i>	+	++	+	++	+++	+++	+++	+++
<i>Rhizopus oryzae</i>	+	+	+	++	+++	+++	+++	++

Note : + = grow <50%  
 ++ = grow 50 – 100%  
 +++ = grow 100%

It has been mention above that the microbe which belong to a probiotic strain must tolerance to conditions of the digestive tract, antibacterial factors, and safety. All potencies are needed to perform its function as a candidate of probiotic (Zahirotul, 2005: Natalia and Priadi, 2006; Sukrisni, 2007: Murwani, 2008). The most potential probiotic strain in this research was *Chrysonilia crassa*. In fact *Chrysonilia crassa* has also the latter character, those has been proved by David and Davis (2000). Their statement was this type of fungi has been used for the manufacture of food and beverages, such as making oncom in West Java and also making oriental foods (koji). This fungi also has been used as a chicken feed additive of the indigenous chicken and shown that the fungi safe for the chicken (Yudiarti, 2012b).

### Conclusion

Species which belong to the most potential to less potential as a probiotic strain were *Chrysonilia crassa*, *Mucor circinelloides*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Mucor sp* *Aspergillus niger*, and *Aspergillus fumigatus*.

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