

Proceeding

INVESTING IN FOOD QUALITY, SAFETY & NUTRITION

Editor:

Lilis Nuraida
Purwiyatno Hariyadi
Ratih Dewanti-Hariyadi
Harsi D. Kusumaningrum
Desty Gita Pratiwi
Nelis Immaningsih



**SEAFast
CENTER**

Southeast Asian Food & Agricultural Science & Technology (SEAFast) Center
Bogor Agricultural University

ISBN: 978-979-16216-8-7

INVESTING IN FOOD QUALITY, SAFETY AND NUTRITION

International Conference Proceeding
Investing in Food Quality, Safety & Nutrition:
Lessons Learned from Current Food Crisis
Jakarta, October 27-28, 2008

Organized by:

Southeast Asian Food & Agricultural Science & Technology (SEAFAST) Center
Bogor Agricultural University

Norman Borlaug Institute for International Agriculture
Texas A&M University

Supported by:

United States Department of Agriculture (USDA)

Ministry of Agriculture, Republic of Indonesia

Indonesian Association of Food Technologists (PATPI)

Editor:

Lilis Nuraida

Purwiyatno Hariyadi

Ratih Dewanti-Hariyadi

Harsi D. Kusumaningrum

Desty Gita Pratiwi

Nelis Immaningsih

Southeast Asian Food & Agricultural Science & Technology (SEAFAST) Center,
Bogor Agricultural University
2009

Investing In Food Quality, Safety and Nutrition

International Conference Proceeding
Investing in Food Quality, Safety & Nutrition:
Lessons Learned from Current Food Crisis
Jakarta, October 27-28, 2008

Editor

Lilis Nuraida
Purwiyatno Hariyadi
Ratih Dewanti-Hariyadi
Harsi-D. Kusumaningrum
Desty Gita Pratiwi
Nelis Immaningsih

Publisher

Southeast Asian Food Science and Technology (SEAFast) Center,
Bogor Agricultural University
Bogor-Indonesia, 2009

National Library Republic of Indonesia
ISBN 978-979-16216-8-7

Acknowledgment

*Thanks to Kamalita Pertiwi, Leo Wibisono Arifin, Yesica Dwi Ariesta, Kandi Jelita,
Virna Berliani Putri, Zulaikhah and Nurwandi
for preparing manuscript of this proceeding*

Copyright©2009

Southeast Asian Food & Agricultural Science & Technology (SEAFast) Center,
Bogor Agricultural University
Kampus IPB Darmaga, Bogor 16680
www.seafast.ipb.ac.id

Roles of Consumers

Consumer Behavior towards Choices & Its Consequences on Nutritional Status	87
<i>John Palmer</i>	

Improving Food Safety & Quality

Food Safety Policy in Indonesia	95
<i>Dedi Fardiaz</i>	
Improving of Food Safety and Quality of SMEs in Indonesia: lesson learned	103
<i>Steven Gregory</i>	
Assuring Indonesian Seafood Quality and Safety: Lessons from the past for a better future	105
<i>Achmad Poernomo</i>	
Use of Simple Micro-titer Plate Assay for Assessment of Biofilm-Forming Bacteria in High Risk Area of Frozen Seafood Plant	115
<i>Damkerng Bundidamorn and Sudsai Trevanich</i>	
Isolation and Identification of Coliforms and <i>Escherichia coli</i> in Frozen Ready to Eat Food under Long Term Storage	121
<i>Pornrujee Suppadit and Sudsai Trevanich</i>	
Growth Inhibition of Contaminated Microbial Spores in Pasteurized Milk by Tea Polyphenol Extract.....	127
<i>Ornurach Uasiriphan and Arunsri Leejeerajumnean</i>	
Migration and Contamination of Polyglycerol Acetate as Alternative Plasticizers in Polyolefin Thermoplastic Matrices in Contact with Water and Olein-Oil Media	139
<i>Basuki Wirjosentono, Hankelman Sarumaha and Marpongahtun</i>	
The Role of Cisadane – Serpong Water Treatment Plant to Ensure 24 – Hour- Drinking Water Supply	149
<i>Audrey Caron Rumamby</i>	
Using Organic Acids, Sodium Hypochlorite And Ozone For <i>Listeria monocytogenes</i> Reduction In Fresh-Cut Carrots	161
<i>Phunnathorn Phuchivatanapong and Arunsri Leejeerajumnean</i>	
Influence of Combination of Alginate, Carrageenan, and Guar Gum as Stabilizing Agents on Ice-Cream Quality	167
<i>Murdinah, Liana Etika Sari, and Anna Muawanah</i>	

Analysis and Planning of Garbage Treatment in a Drinking Water Treatment Plant.....	179
<i>Lidia Khosmatika</i>	
A Study of Cisadane River Based on the Trace Result of PT. Tirta Cisadane).....	193
<i>Hartini Adjam</i>	
Role of Students in Sustaining Food Safety in Campus: A Case Study in "Food Sellers Mentoring" Program in Bogor, Indonesia	203
<i>Galih Nugroho and Kamalita Pertiwi</i>	
Inhibition of <i>Aspergillus parasiticus</i> Growth and Reduction of Aflatoxin by Yeast Isolated from Ragi, an Indonesian Traditional Culture Starter ...	211
<i>R. Dewanti-Hariyadi, D. S. Raharjanti, C.C. Nurwitri and E. Kusumaningtyas</i>	

Improving Competitiveness of Traditional Foods

Policy on Development of Traditional Foods.....	227
<i>Arman Moenek</i>	
Empowerment of Farmers and SMES of Traditional Foods: Lesson Learned	233
<i>Mary Astuti</i>	
Product Development of Traditional Food "Yangko" through Value Engineering	247
<i>Nur Edi Nomalisa, Wahyu Supartono, Darmawan Ari Nugroho, and Anggoro Cahyo Sukartiko</i>	
Sanitation and Hygiene of "Cincau" (Indonesian Traditional Food) Manufacturer.....	255
<i>Dina R. Pangestuti, Laksmi Widajanti, and M. Zen Rahfiludin</i>	
Irradiation to Ensure The Safety and Shelf-Life Extension of Traditional Ready to Eat Meals: Arem-Arem	265
<i>Z. Irawati, C. M. Nurcahya, and I. Lubis</i>	
Effect of Turmeric Extracts (<i>Curcuma domestica</i> L.) on Water Activity Value, Total Microbe and the Number of Coliform of Oven-dried Abon During Storage.....	277
<i>Priyo Bintoro V., Sutaryo and Warsiti</i>	
Application of Herbs and Spices Extracts As Preservatives for Wet Noodles	285
<i>Lilis Nuraida, Nuri Andarwulan, Meilina Sukmawati, and Elvina Yohana</i>	

Improving Food Security

Research Policy on Food Diversification in Indonesia.....	313
<i>Amin Soebandrio</i>	
Local Economy Empowerment and Food Security: Lesson Learned	315
<i>Dahrul Syah</i>	
Optimizing Food Security through Bioavailability Indices	331
<i>Indah Epriliati</i>	
Improvement of Sago Competitiveness for Food Security in Maluku	343
<i>Wardis Girsang and Eddy Ch. Papilaya</i>	
Development of Instant Corn as Raw Material for Traditional Corn-Based Foods: an Effort to Support the Food Diversification Program.....	361
<i>Meta Mahendradatta, Abu Bakar Tawali, Amran Laga</i>	
Research and Development in Processing Technologies of Corn Noodle to Support National Food Security Program	371
<i>Feri Kusnandar</i>	
Industrialization of Modified Cassava Flour (MOCAL/MOCAP) through Cluster Industrial Concept: from Opportunity Identification to Market Development	379
<i>Achmad Subagio, Wiwik Siti Windrati, and Yuli Witono</i>	
Study On Noodle Making From Corn and Sago Flours.....	387
<i>Mariyati Bilang</i>	
Development of Non-Oilseed Legumes as a Source of Protein to Strengthen Food Security in Marginal Areas	397
<i>Achmad Subagio, Wiwik Siti Windrati, Yuli Witono and A. Nafi'</i>	
Consumption and Preference Survey on Maize Based Food Product in Sub-Urban Area and Production area of Maize: Case Study in Bogor and Bojonegoro	405
<i>Harsi D. Kusumaningrum and Aldilla S. Utami</i>	

Improving Nutrition

Public-private Partnership Initiatives to Improve Community Nutritional Status.....	415
<i>Hardinsyah</i>	
Control of Blood Glucose Level by Green Tea and or Mullberry Leaf Tea on Diabetic Rats	417
<i>Evy Damayanthi, Rusman Efendi, Lilik Kustiyah, and Nastiti Kusumorini</i>	

The Impact of Supplementary Feeding Program on Nutritional Status & Academic Performance of University Students	425
<i>Budi Setiawan, Dodik Briawan, Rizal Damanik, Tjahja Muhandari, Dias Indrasti</i>	
Evaluating the Stability of Lutein as a Functional Ingredient in reconstituted UHT Milk.....	457
<i>Dase Hunaefi, Hilton Deeth and Sapna Kamath Voderbet</i>	
Potency of Pegagan (<i>Centella asiatica</i>) as Braintonic to Improve Intelligence of Young Generations in Indonesia	465
<i>Astrisia Artanti and Diana Lo</i>	
The Effect of Food-Based Micronutrient Intervention on the Body Weight Gain, Anemia Prevalence, Ferritin Depletion and Vitamin A Deficiency of Pregnant Woman	473
<i>Nurheni Sri Palupi, Made Astawan, Hadi Riyadi, Ahmad Sulaeman, Prihananto</i>	

Inhibition of *Aspergillus parasiticus* Growth and Reduction of Aflatoxin by Yeast Isolated from *Ragi*, an Indonesian Traditional Culture Starter

R. Dewanti-Hariyadi^{1,2}, D. S. Raharjanti³, , C.C. Nurwitri²
and E. Kusumaningtyas⁴

¹SEAFast Center, Bogor Agricultural University

²Department of Food Science & Technology, Bogor Agricultural University

³Food Science Study Program, Graduate School, Bogor Agricultural University

⁴Research Institute for Veterinary Science (Balitvet), Bogor

email : dewanti@ipb.ac.id

Abstract

Aflatoxins are secondary metabolites of *Aspergillus flavus* and *A. parasiticus* frequently found to contaminate both food and feed. The toxins have been found to be carcinogenic and their presence in food or feed is strictly regulated. Indonesia's climate with high humidity, temperature and amount of rainfall are supportive of mold growth and hence aflatoxin production. Control of aflatoxin production can be achieved by using competitor microorganisms such as mold and yeast. The objective of this research was to isolate yeasts capable of reducing aflatoxin from *ragi*, an Indonesian traditional culture starter and to evaluate the yeast or its metabolite's activity in inhibiting *A. parasiticus* growth, aflatoxin synthesis and degradation of preformed aflatoxin.

The research showed that *all* isolates reduced aflatoxin production by *A. parasiticus*. *Saccharomyces* sp. of *ragi* NKL gave the highest reduction of aflatoxin (AF) production, i.e. 95.9% for aflatoxin B1 (AFB1); 97.1% for aflatoxin B2 (AFB2); 89.4% for aflatoxin G1 (AFG1); 99.1% for AFG2 and 98.1% for total aflatoxin. The metabolites of the *Saccharomyces* sp. also decreased aflatoxin production by *A. parasiticus*. The yeast also showed ability to degrade preformed AFB1, AFB2, AFG1, AFG2 by 36.4 %; 55.6 %; 37.8 %; and 46.7 %, respectively.

Introduction

Aflatoxins are secondary metabolites produced by mold such as *Aspergillus flavus* and *A. parasiticus*. The molds are frequently found to contaminate both food and feed and may produce the toxin to a harmful level.

The toxins have been reported as class 1 carcinogens and their presence in food or feed is strictly regulated.

Indonesia's tropical climate with high humidity, temperature and amount of rainfall are supportive of mold growth and hence aflatoxin production. The mold could grow during the plantation and may also enter during uncontrolled storage of some cereals and nuts. Several studies had reported high prevalence of aflatoxin in peanut (Dharmaputra *et al.*, 1991) and maize (Roedjito *et al.*, 1994) in Indonesia.

Control of aflatoxins in food can be carried out using physical, chemical and biological approach. Due to their resistant to heat, heating is not an effective mean of reducing the toxins. Chemical treatment to reduced aflatoxin using ammonium has been reported by yielded unacceptable food by sensory evaluation. Biological control of aflatoxin using molds or yeast that competes with aflatoxin producer has been reported, such as the use of "afla-guard" in peanut plant (Gardener, 2005).

In Indonesia, several traditional culture starters, called *ragi*, are commonly used in producing various fermented food. Such *ragi* is usually composed of mixtures of molds and yeasts. These microorganisms are potentially capable of competing with *A. flavus* or *A. parasiticus* thus can be used for controlling aflatoxin production in food. Since *ragi* has been used as food ingredient, the mold and or yeasts contained in *ragi* are generally non toxigenic.

Objectives

The objectives of this research were to isolate yeasts capable of reducing aflatoxin production by *A. parasiticus* from *ragi*, evaluate yeast and its metabolites' ability to inhibit *A. parasiticus* growth and aflatoxin synthesis as well as determine its ability to reduce preformed aflatoxin.

Materials and Methods

Material used for obtaining the yeast isolates was *ragi*, an Indonesian traditional dry starter culture to make *tape* (fermented glutinous rice). Various brands of *ragi* were obtained from traditional markets in several cities in Java, i.e. Jakarta, Bandung, Semarang, Rembang, Yogyakarta and Madiun. A toxigenic mold, *Aspergillus parasiticus* F 010 was obtained from Balitvet Culture Collection (BCC), Research Institute for Veterinary Science (Balitvet), Ministry of Agriculture, Bogor). Media used for this study were Potato Dextrose Agar (PDA, Difco), Acidified Potato Dextrose Agar (APDA), Potato Dextrose Broth (PDB, Difco), Malt Extract Broth (MEB, Difco), Peptone (Difco), and Yeast Nitrogen Base, Corn Meal Agar (Difco), Czapek Yeast Extract Agar, Malt Extract Agar dan 25 Glycerol Nitrate Agar.

The research consisted of the following steps: (1) isolation and identification of yeasts isolated from *ragi*, (2) selection of a yeast isolate based on its ability in reducing aflatoxin production by *A. parasiticus*, (3) evaluation of the effect of selected yeast on *A. parasiticus* growth, (4) evaluation of the effect of the yeast metabolites on *A. parasiticus* growth and aflatoxin production by *A. parasiticus*, (4) evaluation of effect of the yeast isolate on preformed aflatoxin. Figure 1 describes the outline of the research.

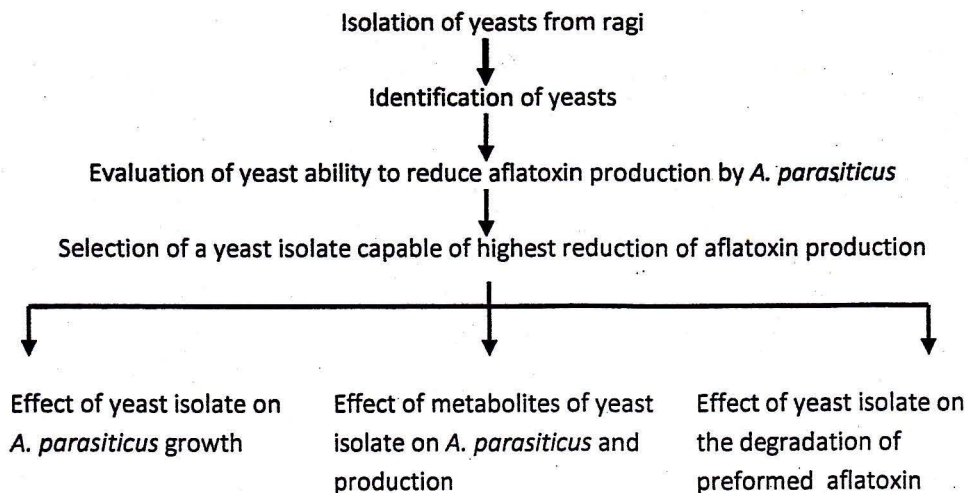


Figure 1. Outline of the research

Isolation of Yeasts from Ragi

Isolation of yeasts was carried out according to Daulay (1989), in which 5 g of ragi is plated onto APDA.

Identification of Yeast

Yeast identification consisted of morphological examination, physiological testings including fermentation and assimilation of seven types of sugar growth at 37°C and their ability to produce extracellular polysaccharide. Results of morphological observation and physiological testings were matched with yeast characteristics according Lodder (1974) and Barnett *et al.* (2000).

Yeast Culture Preparation

Yeast isolates were grown in PDA slants for 2 day, and cell suspension was made by addition of sterile distilled water. Cell number was enumerated with hemacytometer and its concentration was adjusted to ca. 10^6 CFU/g.

Selection of Yeast Isolates for Reduction of Aflatoxin Produced by *A. parasiticus* (Sardjono *et al.* 1992 with some modification)

A half milliliter of *A. parasiticus* spores (ca 10^6 spores/ml) was mixed with 0.5 ml of each yeast isolate suspension (10^6 cells/ml). The mixture was then inoculated onto 100 ml of PDB and incubated for 10 days at room temperature. As a control, a half milliliter of *A. parasiticus* spores (10^6 spores/ml) was inoculated onto PDB medium and incubated for the same period. The amount of aflatoxin produced by the mold was analysed using thin layer chromatography (TLC). The amount of aflatoxin produced by *A. parasiticus* in the presence of yeast was then compared to that of *A. parasiticus* only. Yeast isolate causing the most reduction of aflatoxin produced was selected for further studies.

Effect of Yeast Isolate on *A. parasiticus* Growth (modified from Gourama and Bullerman 1995)

Approximately 0.5 ml of spore suspension of *A. parasiticus* (10^6 spores/ml) was mixed with 0.5 ml of yeast cultures (10^6 CFU/ml) and then inoculated into 100 ml of MEB. The mold and yeast mixtures in MEB was

incubated at room temperature. As a control, 0.5 ml of spore suspensions of *A. parasiticus* (10^6 spores/ml) or 0.5 ml yeast suspension (10^6 CFU/ml) is independently inoculated into MEB medium, and was also incubated at room temperature. Samples of the mixtures and individual mold or yeast were taken and samples were plated on PDA at days 0, 3, 6 and 9. The number of colonies of *A. parasiticus* and yeast was enumerated. At day 9, slide cultures were made and direct interaction between the yeast and the mold was observed using light microscope.

Direct Interaction of Yeast and *A. parasiticus* (modified from Chan dan Tian 2005)

Onto a Petridish containing solidified PDA, place agar (diameter of 5 mm) containing 3 days old mycellia of *A. parasiticus* and then incubated for 72 h at room temperature. A drop (50 μ l) of yeast suspension (10^8 cells/ml) was then placed onto the edge of the mycellium, and incubated for 48 h. Yeast colonies growing on the Petri dish was washed with running water for 2 min and then were then observed using light microscope.

Effect of Yeast Metabolites on *A. parasiticus* Growth and Aflatoxin Synthesis (modified from Sardjono *et al.* 1992)

Yeast metabolites was prepared by growing the yeast isolate in PDB for 7 d at room temperature. Yeast cells were separated by centrifugation at 4000 rpm for 15 min, and supernatant containing metabolites was passed through a filter with pore size of 0.2 μ m in diameter. Yeast metabolites was then inoculated with 0.5 ml spore suspension of *A. parasiticus* (10^6 spores/ml) and was incubated at room temperature for 12 d. As a control, 0.5 ml of *A. parasiticus* spore (10^6 spore/ml) was inoculated into MEB in the absence of yeast metabolite. Aflatoxin concentration and the dry weight of the mycellium were measured after 3, 6, 9 and 12 days of incubation at room temperature.

Effect of Yeast Isolates on the Degradation of Preformed Aflatoxin (modified from Sardjono *et al.* 1992)

Aflatoxin of *A. parasiticus* was produced by growing 7 days old of *A. parasiticus* in PDB and incubate it for 7 days at room temperature. Supernatant was separated from the mycellium and then passed through a filter with pore

size of 0.2 μm in diameter. Supernatant containing aflatoxin was mixed with MEB medium (1:1) and 0.5 ml of yeast cell suspension was inoculated into the aflatoxin-containing MEB. The medium was then incubated for 12 d at room temperature. As a control, a mixture of supernatant containing aflatoxin and MEB medium without addition of yeast cell suspension was incubated at the same incubation time and temperature. Aflatoxin concentration was measured at day-0, 3, 6, 9 and 12.

Aflatoxin Extraction (Heathcot 1984)

Ten milliliters of supernatant was added with 10 ml of chloroform and then homogenized in a blender for 2 min and shaken for 30 min. The mixture was placed in a separation bottle such that two layers were formed. The lower layer containing aflatoxin was filtered through anhydrous sodium sulphate, and the extract was evaporated using a rotavapor. The remaining residue was solubilized in chloroform and was used for aflatoxin analysis.

Aflatoxin Analysis (AOAC 1995)

Analysis of aflatoxin was carried out with one dimension Thin Layer Chromatography (TLC) using chloroform : acetone (9 : 1) as the mobile phase.

The TLC plate used was Silica Gel 60 (Merck). Identification step was done by spotting sample and standard solutions on the TLC plate. The plate was placed in a chamber containing mobile phase and when the spot has stopped moving, the plate was then air dried. Results of the elution was observed under uv light at 365 nm. The R_f (*Rate of Flow*) values of samples and standards were compared and aflatoxin was determined as positive when the R_f of the sample was similar to that of the standard. The aflatoxin concentration was enumerated by comparing fluorescence intensity of samples with those of aflatoxin standards using the following formula :

$$\text{Aflatoxin (ppb)} = \frac{S \times Y \times V \times F_p}{W \times Z}$$

S : Volume of aflatoxin standard (μl) giving fluorescence equivalent to Z μl of sample

- Y : Aflatoxin standard concentration ($\mu\text{g/ml}$)
 Z : Volume of sample extract that gives fluorescence equivalent to S μl aflatoxin concentration
 V : Volume of solvent used to solubilize final sample extract (μl)
 W : Volume of sampel (ml)
 Fp : Dilution factor

Measurement of Dry Weight of Mold Mycellium (Gourama dan Bullerman 1995).

The mold mycellium was filtered using vacuum filtration, washed twice in distilled water and dried with oven at 105°C until constant weight was obtained.

Results and Discussions

Isolation of Yeasts from *Ragi* and Effect of the Yeasts on Aflatoxin Reduction

Three morphologically different yeasts were isolated from samples of *ragi*. Identification step showed that they belonged to the Genera *Saccharomycopsis* and *Saccharomyces*. The ability of isolates in reducing aflatoxin production by *A. parasiticus* varies and is shown in Table 1.

Saccharomyces sp. isolated from Ragi NKL has the highest aflatoxin reduction capacity. The isolate decreased aflatoxin production by *A. parasiticus* by 95.9%, 97.1%, 99.1% and 98.1% for AFB1, AFB2, AFG1 and AFG2, respectively. *Saccharomyces* of *ragi* NKL was then selected for further studies to determine the mechanism of aflatoxin reduction.

Table 1. Aflatoxin reduction by yeasts isolated from *ragi*

Yeast	Brand of <i>Ragi</i>	Aflatoxin reduction (%)				
		AFB1	AFB2	AFG1	AFG2	Total
<i>Saccharomycopsis</i> sp.	Berlian	71.6	85.9	89.4	89.7	86.2
<i>Saccharomycopsis</i> sp.	Gedang	77.1	77.5	68.7	64.3	72.4
<i>Saccharomyces</i> sp.	NKL	95.9	97.1	89.4	99.1	98.1

Effect of *Saccharomyces* on *A. parasiticus* Growth

Figure 2 showed the effect of the yeast isolate on the growth of *A. parasiticus*. *Saccharomyces* sp. caused three logs cycle reduction of *A. parasiticus* growth at three days of incubation. Inhibition of the mold growth was likely caused by nutritive competition between *Saccharomyces* sp. and *A. parasiticus*, since *Saccharomyces* sp. had shorter generation time than that of *A. parasiticus*. Dharmaputra *et al* (2003) stated that fungi with faster growth than *A. flavus* would be potential to control the growth of *Aspergillus flavus*.

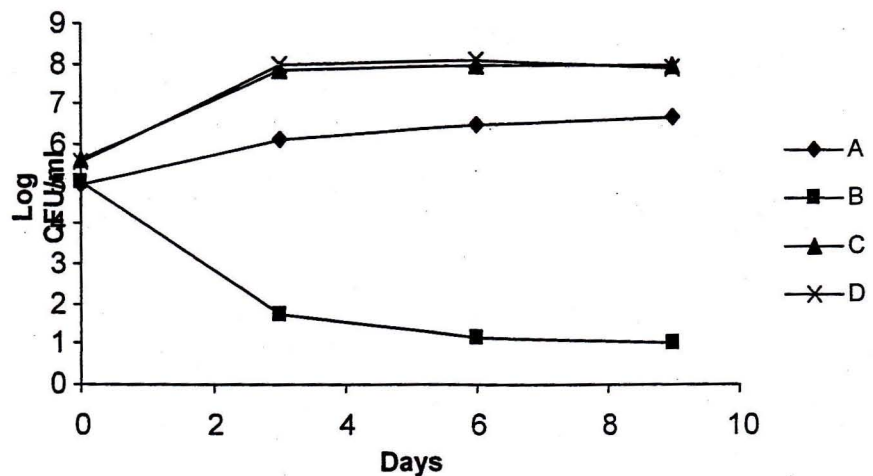


Figure 2. Inhibition of *A. parasiticus* growth by *Saccharomyces* sp. ; A. Number of *A. parasiticus* in the absence of competitor; B Number of *A. parasiticus* in the presence of ; C Number of *Saccharomyces* sp. in the presence of *A. parasiticus* ; D Number of *Saccharomyces* sp. in the absence of mold.

The yeast isolated from ragi also influence the physical appearance of the aflatoxin producing mold. Observation of the morphology of *A. parasiticus* growing in the presence of *Saccharomyces* sp. using light microscope showed that the vesicle size of the mold was smaller and the number of phyalide were less than those of control) (Figure 3). The observation was made at the end incubation (ninth day).

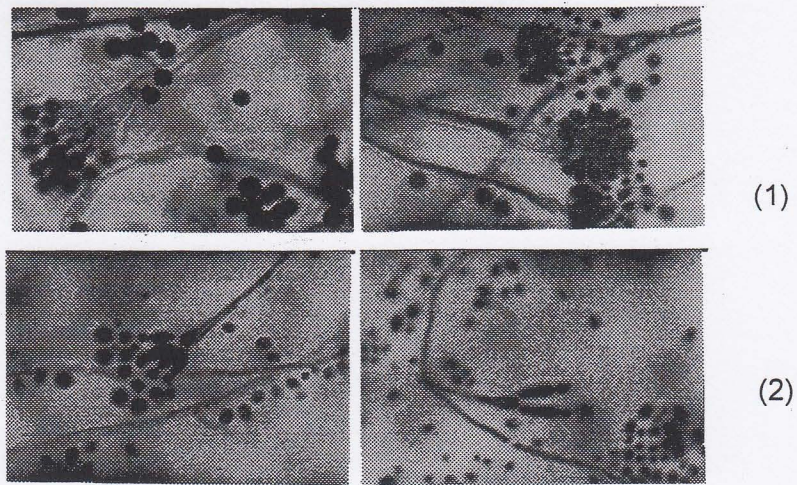


Figure 3. Effect of *Saccharomyces* sp. on *A. parasiticus* morphology after 9 days incubation (1) *A. parasiticus* (2) *A. parasiticus* together with *Saccharomyces* sp. (magnification 1000x)

β -glucanase enzyme produced by *Saccharomyces* sp. has been reported to destroy cell wall of *A. parasiticus*. This could be accounted for the morphological changes of *A. parasiticus* when it was grown in the presence of the yeast. The microscopic observation also showed that *Saccharomyces* sp. adhered to *A. parasiticus* hypha as presented in Figure 4.

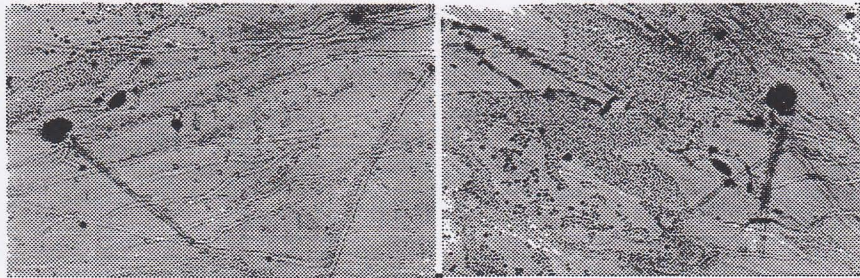


Figure 4. Microscopic observation showing direct interaction between *Saccharomyces* sp. and *A. parasiticus*

Effect of Yeast Metabolites on *A. parasiticus* growth and Aflatoxin Biosynthesis

The metabolites of *Saccharomyces* sp. isolated from ragi had inhibition activity on *A. parasiticus* growth. This research showed that *A. parasiticus*

grown in a medium containing yeast metabolite yielded a lower mass as indicated by the lower weight of the mycelium as compared to that grown alone in a medium (Figure 5).

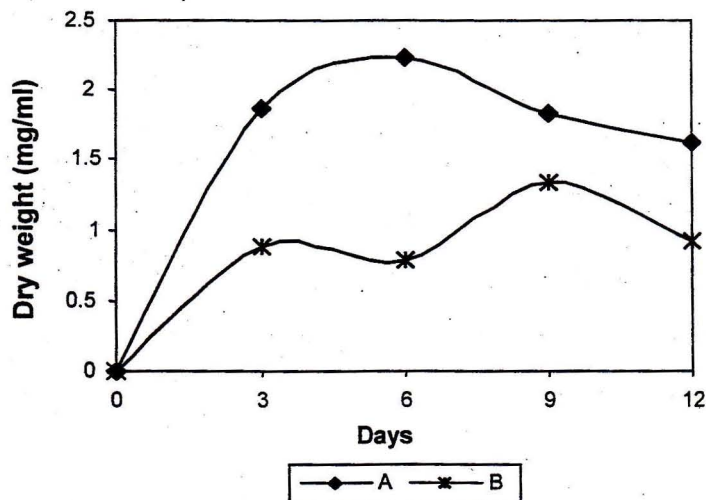


Figure 5. Effect of *Saccharomyces* sp. metabolites on *A. parasiticus* growth; A *A. parasiticus* control; B *A. parasiticus* growing in medium supplemented with *Saccharomyces* sp. metabolites

In addition, *Saccharomyces* sp. metabolites also inhibited aflatoxin biosynthesis by *A. parasiticus*. The yeast isolated from *ragi* inhibited all types of aflatoxins, i.e. aflatoxin B1, B2, G1 and G2 (Figure 6). This observation was similar to Purwijantiningsih (2005) who reported that *Candida* sp. metabolites inhibited *A. flavus* growth and aflatoxin biosynthesis by the mold.

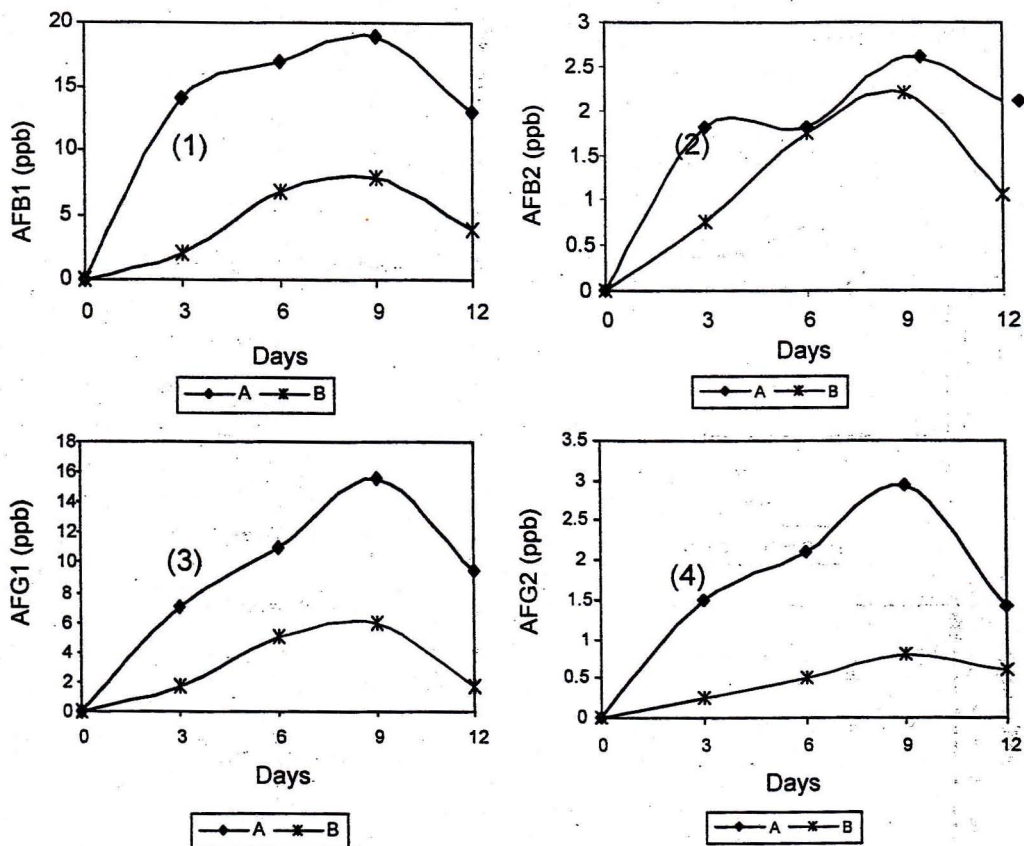


Figure 6. Effect of *Saccharomyces* sp. metabolite on biosynthesis of aflatoxin (1) B1 (2) B2 (3) G1 (4) G2: **A** *A. parasiticus* growing in the absence of yeast metabolites; **B** *A. parasiticus* growing in medium supplemented with yeast metabolites

Effect of *Saccharomyces* sp. on the Degradation of Preformed Aflatoxin

Degradation of aflatoxin was studied by inoculating *Saccharomyces* sp. into medium containing preformed aflatoxin. The results showed that *Saccharomyces* sp. decreased the amount of preformed aflatoxin by 44.88% (Figure 7).

Degradation of aflatoxin could be caused by aflatoxin binding by other microorganisms or their components.. Mannan and glucan of the cell wall of *S. cerevisiae* cell have been reported to bind and reduce several mycotoxins, such as aflatoxin B1, zearalenon, fumonisins and deoxynivalenol by 95%, 77%, 59%

and 12 % consecutively (Galvano *et al* 2001). Aflatoxin reduction by other fungi has been reported by Nakazato *et al.* (1990).

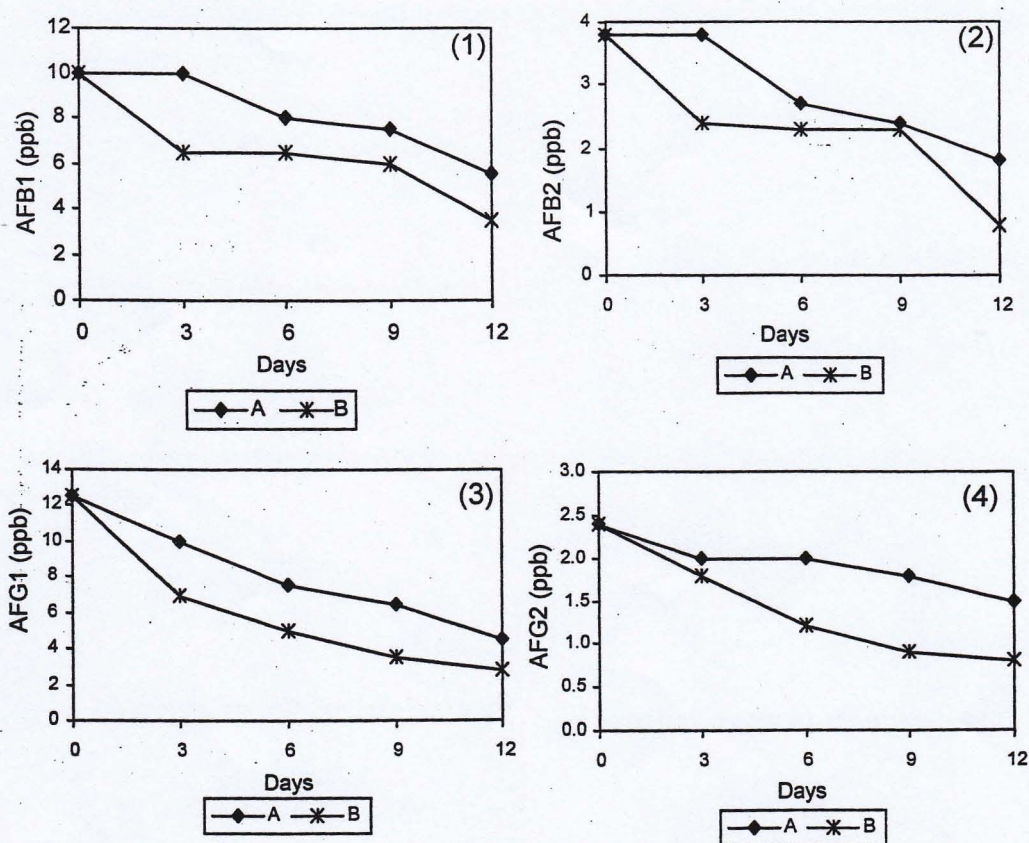


Figure 7. Degradation of preformed aflatoxin (1) B1 (2) B2 (3) G1 (4) G2 by *Saccharomyces* sp.

Conclusions

Several yeasts belonging to Genera of *Saccharomyces* and *Saccharomycopsis* isolated from *ragi* possessed ability to reduce aflatoxin produced by *A. parasiticus*. *Saccharomyces* sp. from *ragi* NKL was able to reduce aflatoxins of *A. parasiticus* by 98,1%. The isolated yeast also inhibited *A. parasiticus* growth, as shown by the decrease in mold growth, smaller vesicles and lower number of phyllids. Reduction of aflatoxin by *Saccharomyces* was also due to its metabolites. The yeast metabolites were able to inhibit *A.*

parasiticus growth and aflatoxin biosynthesis. *Saccharomyces* sp. also capable of degradation of preformed aflatoxin and reduced preformed aflatoxin by 44.88%.

References

- [AOAC] Association of Official Analytical Chemist. 1995. Natural Poison. In: Hoewitz W (ed.). Official Methods Analysis of The Association of Official Analytical Chemist. Edisi 11. Washington DC : Association of Analytical Chemist
- Barnett JA, Payne RW, Yarrow D. 2000. *Yeast Characteristic and Identificationnnnn*. 3rd ed. Cambridge : Cambridge University Press
- Chan Z, Tian S. 2005. Interaction of antagonistic yeasts postharvest pathogens of apple fruit and possible mode of action. *Postharvest Biology and Technology*. 36 : 215-223
- Daulay D. 1989. Identification of Microorganisms in Tauco Fermentation Research Report. Food Microbiology Laboratorium, PAU Pangan dan Gizi. Bogor Agricultural University (in Bahasa Indonesia).
- Dharmaputra OS, Tjitrosomo HSS, Susilo H, Sulaswati 1991. *Aspergillus flavus* and aflatoxin of peanuts collected from three markets in Bogor, West Java, Indonesia. Proceedings of the 12th ASEAN Seminar on Grain Postharvest Technology, Surabaya, Indonesia, 29-31 Agustus 1989 : 110-123
- Dharmaputra OS, Putri ASR, Retnowati I, Saraswati S. 2003. Use of *Trichoderma harzianum* to control aflatoxin producoing *Aspergillus flavus* in peanut *Jurnal Fitopatologi Indonesia* 7(1) : 28-37 (in Bahasa Indonesia)
- Galvano F, Piva A, Ritieni A, Galvano G. 2001. Dietary strategies to counteract the effect of mycotoxin : A Review. *J. Food Prot.* 64: 120-131
- Gourama H, Bullerman LB. 1995. Inhibition of growth and aflatoxin production of *Aspergillus flavus* by *Lactobacillus* species. *J. Food. Prot.* 58 : 1249-1256
- Heathcot JG. 1984. Aflatoxin and related toxin. Di dalam : Betina V, editor. *Mycotoxins : Production, Isolation, Separation & Purification*. Amsterdam : Elsevier Sci. Publ. hlm 89-126
- Lodder J. 1974. *The Yeast : A Taxonomic Study* 2nd ed. Amsterdam : North-Holland Publ. Comp

- Nakazato M, Morozumi S, Saito K, Fujinuma K, Nishima T, Kasai N. 1990. Interconversion of aflatoxin B1 and aflatoxicol by several fungi. *Appl. Environ. Microbiology*. 56(5) : 1465-1470.
- Purwijantiningsih E. 2005. Inhibition of Growth and Aflatoxin Production by *Aspergillus flavus* by Fungi isolated from *Ragi Tempe*. Thesis. Sekolah Pascasarjana., Bogor Agricultural University (in Bahasa Indonesia)
- Roedjito D, Anwar F, Damayanthi E. 1994. Studies on Aflatoxin Content of Cereal, Nuts and their Products in Markets and Household. *Direktorat Pembinaan Penelitian dan Pengabdian Masyarakat, Dirjen DIKTI*, Ministry of Education. Jakarta (in Bahasa Indonesia)
- Sardjono, Rahayu K, Sudarmadji S. 1992. Growth and aflatoxin production by *Aspergillus oryzae*. *Asean Food Journal*. 7:30-33