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Mold Contamination and aflatoxin B_1 levels in salted fish commodities from traditional market in Yogyakarta and Surabaya, Indonesia

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ABSTRACT

Aims: To identify mold contaminant on salted fish, from two different market locations (Kenjeran market, Surabaya and Beringharjo market, Yogyakarta). Furthermore, levels of AFB1 (aflatoxin B1) in salted fish samples were assayed.
Methodology and results: The samples were cultivated on DRBC (Dichloran Rose Bengal Chloramphenicol Agar) and DG-18 (Dichloran (18%) Glycerol Agar) medium for enumeration, then transferred on MEA (Malt Extract Agar) medium for isolation and identification, followed by ELISA test to measure the AFB1 level. Meanwhile aflatoxin biosynthesis correlated genes (i.e. *aflR*, *nor-1* and *omtB* genes) were identified using Polymerase Chain Reaction (PCR) method. The results showed that *Aspergillus tamarii* and *A. flavus* being contaminant on salted fish along with *A. sydowii*, *A. niger*, *A. versicolor*, *Penicillium citrinum*, and *P. chrysogenum*. *Rhizopus* sp. contamination was also found. AFB 1 was positively detected in all of samples with the highest concentration measured was 75.81 µg/kg which belong to Lidah salted fish and the lowest concentration measured was 4.33 µg/kg which belong to Rese salted fish. The suspected *A. flavus* and *A. tamarii* isolated from salted fish was positively detected in the presence of *aflR*, *nor-1* and *omtB* genes.
Conclusion, significance and impact of study: Mold contamination was detected in salted fish from two different markets and all of those samples were contaminated by AFB1. These can be important information related to food safety aspect for salted fish.

***Keywords**:* Salted fish, mold contamination, Aflatoxin B1

INTRODUCTION

Indonesia is an archipelago country with the abundant of fish commodities, which are non-perishable due to high moisture and nutrient content of fish. According to the statistical data on Indonesia's fisheries year of 2015, fish production in 2014 reached 6.50 million tons. Furthermore, in Indonesia, the most common method to prolong the self-life of fish is fish salting, which are done by adding salt and drying. In Indonesia, salted fish is the second preference after fresh fish to be consumed. It is also often used as an ingredient or condiment in several Indonesian foods.

According to Sutarni (2013), the variation of salt concentration used in the salting process affects the salt penetration into the fish's body. Although salting the fish may extend the self-life of fish, due to moisture content reduction, the product is often sold uncovered. This can lead to contamination of the product either from dust or insect, such as flies. Sources of contamination also come from poor personal hygiene from both the sellers and the buyers. Based on fact that mentioned before, mold contamination may occur in salted fish product. Moreover, Indonesia's tropical climate which has a high relative humidity also gives a contribution to mold contamination. In addition, based on Indonesia National Standard of salted fish (SNI 01-2721-1992), which mentioned that mold must not be detected.

The mold can grow at temperatures of 20-30 °C, with a relatively low a_w at 0.85 (Rahayu *et al*., 2014) and affects the appearance of salted fish e.g. color and smell. Furthermore, the most negative effect from mold contamination is mycotoxins, because it cannot be easily detected by the eyes. However, the growths of mold and mycotoxins production are dependent on several factors such as the mold strain and competitor, substrates, temperature and relative humidity.

The occurrence of mold contamination has been reported in dried salted fish, dried fish and smoked fish. Smoked fish from tropical area has the potential for

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contamination by toxigenic molds, such as *Aspergillus flavus* (Adebayo-Tayo *et al.,* 2008). *Aspergillus flavus* is also the dominant species identified on smoked fish from Sierra Leone area, followed by contamination of *A.ochraceus*, *A. niger* and *A. tamarii* (Jonsyn and Lahai, 1992). In addition, *A. flavus* is also isolated from dried fish in Sri Lanka and Indonesia (Atapattu and Samarajeewa, 1990). Furthermore, *A. tamarii* is found to be the predominant contaminant in Maldives fish (Mohamed, 2013). *Aspergillus sydowii* is also isolated from dried fish sold in traditional markets in Jakarta (Santoso *et al*., 1999).

Some mold can produce mycotoxins as their second metabolite. Aflatoxin, which majority produced by *A. flavus* and *A. paraciticus,* has hepatotoxic, mutagenic, teratogenic, and carcinogenic effect for both humans and animals (Alberts *et al*., 2006). An outbreak of aflatoxin was first noticed in the early 1960s in the UK (Turkey X disease) (Do and Choi, 2007). Furthermore, according to The International Agency for Research on Cancer (IARC, 2002), AFB1 is classified as a group 1 carcinogen which means carcinogenic to humans. The occurrence of aflatoxin is very common in developing countries (Samuel *et al*., 2009). Moreover, aflatoxin is very heat stable, therefore, they are not easily removed from food, even after processing by high temperature (Rahayu *et al*., 2003). According to Firsvad *et al*. (2005), there are other molds that have the ability to generate aflatoxin. There are *A. nomius*, *A. pseudotamarii, A. parvisclerotigenus*, and *A. bombycis* of section Flavi, *A. ochraceoroseus* and *A. rambellii* from section Ochraceorosei and *Emericella astellata* and *E. venezuelensis* from Nidulatans section.

Because of those reasons, detection and identification of mold contamination on salted fish from traditional market in Indonesia is required as food safety and good manufacturing processes information. The aim of this study was to identify the mold that can contaminate salted fish, and to quantify the AFB1 level in salted fish.

MATERIALS AND METHOD

Sample collection and preparation

A total of 20 different salted fish samples were used in this study and purchased from two different traditional markets to illustrate mold domination from different supply chain. Salted fish from Kenjeran market, Surabaya were sold in the open-air stalls near the production site, while salted fish from Beringharjo market, Yogyakarta were supplied from another city with semi-permanent stalls. All salted fish were produced traditionally by adding salt followed by sun-drying. Each sample was stored in polyethylene plastic with specific code. Samples were stored in a cool room in the microbiology laboratory, Center Study of Food and Nutrition, Universitas Gadjah Mada. Before performing the tests, the salted fish was allowed to heat up to room temperature (±25 °C).

Mold Enumeration

All salted fish samples were inoculated on DRBC media (Oxoid) and DG-18 (Oxoid) directly, large-sized samples were cut with sterile scissors before inoculated. Samples were then incubated at room temperature (±25 °C) for 5 days. Colonies formed were differentiated by appearance and color (i.e. bluish grey, green, brownish green, black, yellow, bluish green and velvet blue colony) for enumeration. Enumeration shows percentage of samples contaminated. The total pieces of analyzed samples can be seen in Table 1.

% of samples contaminated =

$$\frac{(\text{samples contaminated by mold})}{(\text{total pieces of analyzed samples})} \times 100\%$$

Identification of mold contamination

Colonies that grew on the surface of the sample were then isolated based on the colour differences on MEA media (Oxoid). Then the incubation was done for 5 days at room temperature (±25 °C). Isolates obtained were identified using macromorphology and micromorphology according to Rahayu *et al*. (2014).

Determination of Aflatoxin B1 contamination

Detection of AFB1 in salted fish was assayed using ELISA. Twenty-five grams of crushed samples was extracted using 75 mL of 70% methanol and shook for 3 min. There was no pre-treatment to reduce the salt content in salted fish samples. The extract was then separated from the cake using Whatmann paper No.1. AFB1 content was tested in accordance to the manual on RIDASCREEN Aflatoxin B1 ELISA kit (Bioo Scientific) then absorbance at 450 nm wavelength was measured. AFB1 standard solutions concentrations of 0, 2, 5, 20, and 50 ng/mL were used as the calibration curve. The calculated AFB1 in extract was then converted to AFB1 in weight of samples.

Detection of aflatoxigenic genes in *Aspergillus* sp

Molecular detection of aflatoxigenic genes were performed based on PCR method for amplification of several genes correlated to aflatoxin production, such as *aflR*, *nor-1*, and *omtB* genes. Reaction of PCR amplification was performed in 25 µL of mix PCR using Ready To Go PCR kit mixed with 21 µL ddH_2O, 1 µL of each primer, and 1 µL of DNA template. The PCR program of *aflR* gene based on the procedure operated by Rahimi *et al*. (2008), amplification was started with 1 cycle initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min, and extension at 72 °C for 2 min, as well as 1 cycle of final elongation at 72 °C for 10 min. In addition, *nor-1* gene amplification was performed according to Geisen (1996), which consisted of 1 cycle initial

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denaturation at 94 °C for 5 min, 30 cycles of denaturation, annealing, and extension at 95 °C, 1 min; 65 °C, at 2 min; and 72 °C, at 4 min, respectively. While, The PCR program for *omtB* gene was based on Rodrigues *et al*., (2007) comprising of 1 step initial denaturation at 94 °C for 3 min continued by 35 cycles denaturation, annealing, extension for 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C, respectively and 1 cycle final extension for 10 min at 72 °C.

RESULTS AND DISCUSSION

Mold contamination of salted fish

From observation of the incubated samples, it became clear that all of the samples on DRBC and DG 18 media are contaminated by mold as seen on Figure 1. Yeast colonies were also found on the salted fish, but was not identified. Contamination on examined salted fish was dominated by *Aspergillus*, followed by *Penicillium*. Hence, suspected aflatoxin producer was also successfully isolated (Figure 1) and identified as *A. flavus* and *A. tamarii*. This was in alignment with result from Hassan *et al*., (2011) that the most dominant contamination of salted fish which is randomly collected from different shops and retail markets at Giza Governorate was by *Aspergillus* sp. members, at about 83.3% with *A. flavus* contamination being at 66.6%. According to Pitt and Hocking (2009), *Aspergillus* and *Eurotium* were the most dominant contamination species found on dried food in tropical and subtropical regions. *Aspergillus* and *Eurotium* were also dominant on smoked dried fish from warm water regions (Adebayo-Tayo *et al*., 2008). This was caused by *Aspergillus* which usually grows faster than *Penicillium* but takes longer to sporulate (Rahayu *et al*., 2014).

The samples obtained from Kenjeran market Surabaya had different dominant mold from those obtained in Beringharjo market, Yogyakarta. This was due to differences in the supply chain and the handling. Samples from Kenjeran market, Surabaya were sold in coastal and open roadside stalls, in addition the production sites and the dried fish stalls were close to each other. While the samples from Beringharjo market, Yogyakarta consisted of salted fish supplied from Pati, Central Java and the stalls were all in one room, with not only salted fish stalls but also another staple food (i.e. vegetables, fruits, tubers, and beef). According to Sharma (2012), *A. niger* is commonly found in indoor environment, fruit and vegetable. This explains that mold domination in Beringharjo market comes from the environment of the stalls. On the other hand, *Aspergillus* sp. are closely associated with the soil (Owaga *et al*., 2009). Therefore, it is possible that the sun-drying of salted fish on the roadside of Kenjeran market could have exposed with *Aspergillus* sp., particularly from *A. flavus*.

The frequency of mold contamination on salted fish is shown in Table 2. Samples from Kenjeran market, Surabaya showed that all samples were found to have mold contamination. The dominant mold was 100% *A. tamarii* (9/9 samples) and 89% *A. flavus* (8/9 samples). Lidah salted fish had the highest frequency of *A. flavus* contamination which was about 92%. Spores of *A. flavus* were found in the tropical air. In Indonesia itself, which is a tropical country, the drying of salted fish still takes the advantage of sunshine, thus increasing the likelihood of contamination by fungal spores. The samples from Beringharjo market, Yogyakarta were also contaminated by mold. The dominant molds were *A. niger*, *A. flavus*, *A. tamarii*, and *Rhizopus* sp with the frequency of each contamination approximately 45% (5/11 samples). The highest *A. flavus* contamination was found on Pethek salted fish at the level of 50%. By the presence of *A. flavus* on salted fish, it is possible to find aflatoxin contamination.

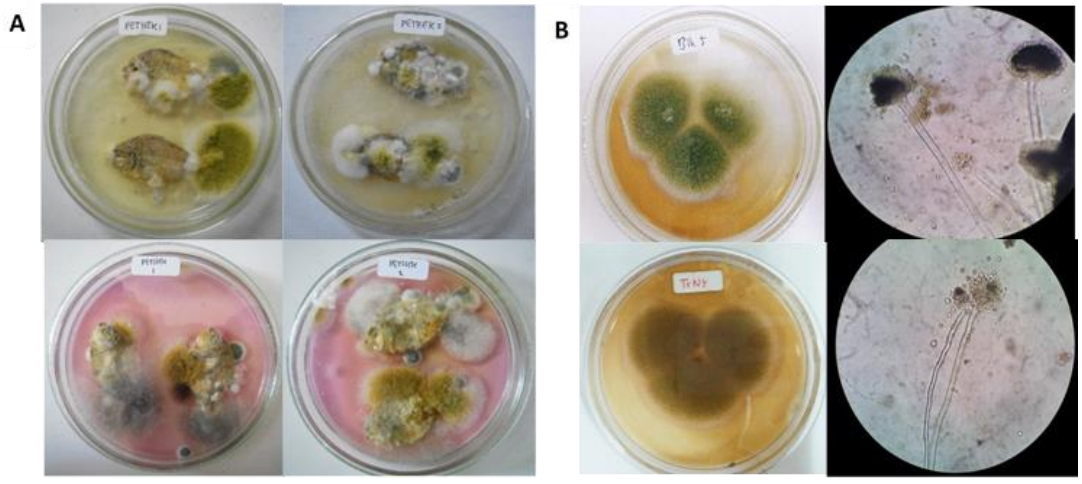

Figure 1: (a) Mold diversity contaminating on salted fish (upper-side is on DG-18 medium and down-side is on DRBC medium). (b) Suspected aflatoxin producer identified as *A. flavus* (upper-side) and *A. tamarii* (down-side).

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Table 1: Macro- and micro-morphology of identified mold.

Isolate code	Macro morphology	Micro morphology	Mold identified
HAF1	Green colony, ±50 mm colony diameter, reverse colony creamish-yellow	Biseriate, spherical vesicle, hyaline, long conidiophores	*A. flavus*
BAAS1	Bluish gray colony, ±15 mm colony diameter, produce exudate, reverse colony brown	Biseriate, circular vesicle, hyaline, cylindric phialides, small conidia	*A. sydowii*
CAT2	Solid brownish green colony, ±50 mm colony diameter, reverse colony creamish-yellow	Biseriate, long conidia, spherical vesicle, hyaline, cylindric phialides	*A. tamarii*
KAV2	White colony at the beginning and turn to yellow, velvet-like appearance, ±10 mm colony diameter, reverse colony cream	Biseriate, oval (spoon-shaped) vesicle, spreading conidia, hyaline	*A. versicolor*
HAN1	Black colony, ±60 mm colony diameter, reverse colony dark creamish-yellow	Biseriate, black spherical vesicle, long conidiophores, circular conidia, cylindric phialides	*A. niger*
HBPC1	Velvet blue colony, ±25 mm colony diameter, reverse colony pale yellow, produce exudate	Tervertisilata, hyaline, cylindric phialides	*P. chrysogenum*
HAPC2	Bluish green colony, ±25 mm colony diameter, produce exudate, slow growth, reverse colony pale yellow	Bivertisilata, hyaline, cylindric phialides	*P. citrinum*

Another important genus which was identified is *Penicillium*, although it had a lower frequency than the *Aspergillus* genus. *Penicillium citrinum* and *P. chrysogenum* were also identified on some salted fish samples. The highest *P. citrinum* contamination was found on Ebi salted fish, at about 50% and the highest *P. chrysogenum* contamination was found on Lidah salted fish, at about 33%. *P. citrinum* produces citrinin toxin which is nephrotic and carcinogenic for animals (Flajs and Peraice, 2009).

Seven isolates identified had halotolerant and xerophilic properties. *Aspergillus sydowii* is a mold mostly found on marine organisms. According to Prakash *et al*. (2011). *Aspergillus flavus* and *A. niger*, which originated from dried seafood products in India showed salt resistance of up to 18%. *Aspergillus flavus* was also found to be the dominant contaminant on smoked dried fish (Adebayo-Tayo *et al*., 2008). In the case of Maldives fish, which is a salted fish from the Maldives region, *A. tamarii* contamination was higher than *A. flavus* contamination, because Maldives fish has a low salinity at an average 2.08% (Mohamed, 2013). It is similar to the total mold contaminant in samples from Kenjeran market, Surabaya.

Aflatoxin B1 contamination in salted fish

The presence of *A. flavus* indicates the possibility of contamination by AFB1 in salted fish. Out of the samples analyzed, all were positively contaminated by AFB1. The highest AFB1 contamination was on the Lidah salted fish taken from Kenjeran market, Surabaya which had 75.81 µg/kg. And the lowest AFB1 contamination was on Rese salted fish taken from Beringharjo market, Yogyakarta which had 4.38 µg/kg. The AFB1 levels of the salted fish are shown in Table 2.

The concentration of aflatoxin in samples was determined from the standard calibration curve plotted in the range of 0, 2, 5, 20, and 50 ng/mL using ELISA. Linear regression analysis was further used for the quantification of aflatoxin present within the samples. The standard calibration graph showed excellent linearity with R^2 value of 0.9967. The limit of detection and quantification of ELISA method were 0.34 and 1.02 ng/mL respectively.

If the result of ELISA test is correlated with the frequency of *A. flavus* contamination on the samples (Figure 2), it can be fully understood that the amount of AFB1 in the samples was not affected by the frequency of *A. flavus* contamination. Hence, if the ELISA result compared to mold species contaminated on samples, not all samples were contaminated by *A. flavus* which is an AFB1 producer. Meanwhile, all of samples were positively contaminated by AFB1 in different amounts.

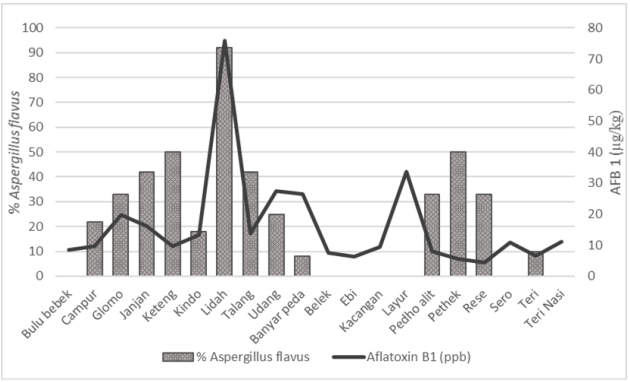

Figure 2. Corellation frequence of *A. flavus* and aflatoxin B1 level.

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Table 2: Frequence of mold contamination and Aflatoxin B1 levels in salted fish samples.

Salted Fish	Species	Analyzed sample	Source	Aflatoxin B1 (µg/kg)	Frequence of mold contamination (%)*							
					A. flavus	*A. tamarii*	*A. niger*	*A. sydowii*	*A. versicolor*	*P. citrinum*	*P. chrysogenum*	*Rhizopus* sp
Bulu bebek	*Thryssa mystax*	8	Kenjeran market, Surabaya (9 samples)	8.52	-	25	-	38	25	-	-	-
Campur	-	18		9.63	22	28	39	-	-	-	-	-
Glomo	*Johnius coitor*	12		19.86	33	50	-	33	-	25	-	-
Janjan	*Anguilla rostrata*	12		16.14	42	25	42	17	33	-	-	-
Keteng	*Mystus planiceps*	12		9.63	50	25	8	50	25	-	-	-
Kindo	Unknown	11		13.29	18	36	-	27	-	-	-	55
Lidah	*Cynoglossus lingua*	12		75.81	92	25	8	25	25	-	33	-
Talang	*Chorinemus tala*	12		13.77	42	50	-	-	-	-	-	-
Udang	*Litopenaeus vannamei*	12		27.42	25	25	8	-	17	-	-	-
Banyar peda	*Rastrelliger kanagurta*	12	Beringharjo market, Yogyakarta (11 samples)	26.37	8	-	8	-	-	-	-	-
Belek	Unknown	12		7.59	-	-	-	-	-	-	8	-
Ebi	*Litopenaeus vannamei*	12		6.24	-	-	-	-	-	50	-	17
Kacangan	*Charangidae sp*	12		9.39	-	-	17	-	-	-	25	-
Layur	*Trichurus savala*	12		33.72	-	8	-	8	-	17	-	8
Pedho alit	*Rastrelliger scombridae*	12		7.98	33	8	-	-	-	-	-	17
Pethek	*Leiognathus equulus*	8		5.61	50	-	-	-	-	63	-	100
Rese	*Acetes indicus*	30		4.38	33	7	7	-	-	-	30	-
Sero	Unknown	12		10.80	-	-	-	-	-	-	-	23
Teri	*Stolephorus tri*	10		6.48	10	-	20	-	10	20	-	-
Teri Nasi	*Stolephorus commersonii*	22		11.19	-	-	9	-	-	-	14	-

*The number expressed as the percentage was calculated in relation to the amount of analyzed samples.

Aflatoxin contamination has no correlation to the frequency of *A. flavus* on samples because it does not grow well. The growth of *A. flavus* was resisted by other more dominant molds, so there were some samples that were not contaminated by *A. flavus*. In addition, according to Abidin *et al*. (2010) *A. tamarii* also can produce AFB1 and AFB2, probably the presence of aflatoxin was produced by *A. tamarii* instead of *A. flavus*. Moreover, the ELISA method has low specificity to detect other components which have a similar structure to AFB1, such as AFB2, AFG1 and AFG2 (Leszczynska *et al*., 2001). The salt content in the samples caused a matrix effect that can affect the absorbance measurement. It is also possible a cross-linking component to occur in the sample, thus affecting the selectivity and specificity of ELISA measurement (Rachmawati *et al*., 2004). Because of those reasons, ELISA method has a limitation for analyzing AFB 1 in salted fish samples, regarding there was no pre-treatment to reduce the salt content in samples.

Molecular detection of aflatoxin biosynthesis genes in *Aspergillus* strain

Fungal group of *Aspergillus* (green Aspergilli and black Aspergilli) found in salted fish were then selected for aflatoxigenic detection based on PCR amplification method. Three types of genes involved in aflatoxin biosynthesis namely *aflR*, *nor-1*, and *omtB* were used in detecting aflatoxin production ability. Based on amplification result, primer *aflR*, *nor-1*, and *omtB* were able to amplify an expected for approximately 600 bp, 400 bp, and 1000 bp fragment in aflatoxigenic isolates, respectively (Figure 3). Aflatoxigenic and nonaflatoxigenic

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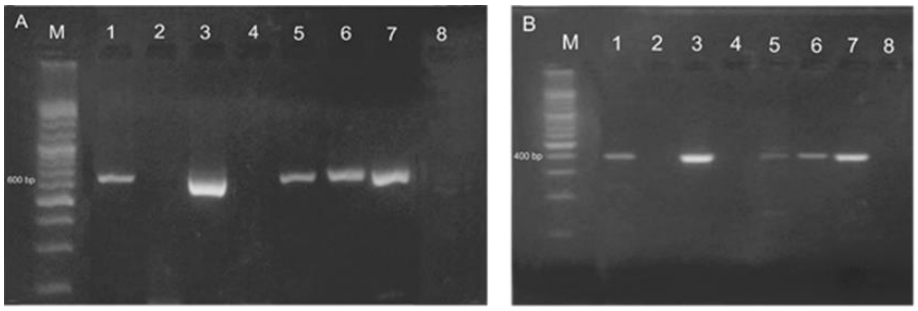
strains could be determined on the basis of amplification of these three target DNA fragments.

Based on Table 3, it demonstrated that almost all isolates of green Aspergilli showed positive results in the presence of *aflR*, *nor-1*, and *omtB* genes. The positive results against those primer were *A. flavus* and *A. tamari*. Meanwhile, none of black Aspergilli have those three genes detected. Two isolates only have both regulatory genes, *aflR* and *nor-1*, while the other two isolates have none of three target genes.

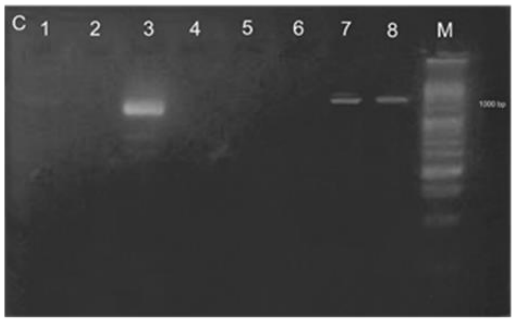
The existing of three genes target involved in aflatoxin biosynthesis such as *aflR*, *nor-1*, and *omtB* may indicate that the isolate was able to produce aflatoxin. PCR analysis was able to amplify aflatoxin biosynthetic genes, i.e *aflR*, *nor-1*, and *omtB* in almost green Aspergilli group, while none of *omtB* gene detected in black Aspergilli. Aflatoxin, a secondary metabolites which is a polyketide-derived, is produced via the following conversion path: acetate polyketide anthraquinones xanthones aflatoxin (Bhatnagar *et al.*, 2003; Yu, 2012). However, aflatoxin formation depends on the final step of aflatoxin biosynthesis pathway. In this case, norsolorinic acid (NOR) is the earliest step and the first stable aflatoxin precursor in the aflatoxin biosynthetic pathway thus it play a role in polyketide synthase (Yu *et al*., 2004; Yu, 2012). In contrast, O-methyltransferase (*omtB*) involve in the later step of aflatoxin formation. Meanwhile, *aflR* is a positive regulatory gene which is required for transcriptional activation of most of the structural genes such as *nor-1* and *omtB* (Yu *et al*., 2004). Thus, the absence of *omtB* gene in such black Aspergilli isolate might indicate that those are unable to produce aflatoxin, while the presence of those three target genes in green Aspergilli are possible to support the correlation of ability in aflatoxin production.

Table 3: Detection of aflatoxigenic and non-aflatoxigenic *Aspergillus* group based on PCR amplified product.

Code	Mold species	*Aspergillus* group	Primer AFB1		
			nor-1	*aflR*	*omtB*
IC1	*A. tamarii*	Green Aspergilli	-	-	-
IC2	*A. tamarii*		+	+	+
IHK1	*A. flavus*		+	+	+
IHK2	*A. flavus*		+	+	+
IB	*A. niger*	Black Aspergilli	-	-	-
IHTM	*A. niger*		+	+	-
IH	*A. niger*		+	+	-
IHIT	*A. niger*		-	-	-



Code : 1: IH; 2: IB; 3: IHK1; 4: IC1; 5: IHTM; 6: IC2; 7: IHK2; 8: IHIT



Code : 1: IH; 2: IB; 3: IHK1; 4: IC1; 5: IHTM; 6: IHIT; 7: IC2; 8: IHK2

Figure 3: Amplified product of five target genes in *Aspergillus*. (A) Amplified product of *aflR* gene in *Aspergillus*; (B) Amplified product of *nor-1* gene in *Aspergillus*; (C) Amplified product of *omtB* gene in *Aspergillus*.

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CONCLUSIONS

Aspergillus tamarii, *A. flavus*, *A. sydowii*, *A. niger*, *A. versicolor*, *P. citrinum*, *P. chrysogenum* and *Rhizopus sp* are identified being contaminant on salted fish. Salted fish samples from Kenjeran market were dominated by *A. tamarii* and *A. flavus*, while salted fish samples from Beringharjo market were dominated by *A. flavus* and *A. niger.* The difference of supply chain from both sources of samples affects the mold domination. In addition, AFB1 was detected positively in all of samples with highest level belong to Lidah salted fish (75.81 µg/kg) and the lowest AFB1 level belong to Rese salted fish (4.33 µg/kg). Besides, ELISA test has a limitation for quantify AFB 1 level from salted fish, due to the high salt content that can be interference for absorbance measurement. The suspected *A. flavus* and *A. tamarii* isolated from salted fish were positively detected in the presence of *aflR*, *nor-1* and *omtB* genes.

REFERENCES

Abidin, M. Z., Ahmad, M. M. and Javed, S. (2010). Advances in molecular detection of *Aspergillus*: An update. *Archives of Microbiology* **192, 409-425.**

Adebayo-Tayo, B. C., Onilude, A. A. and Patrick, U. G. (2008). Mycoflora of smoked dried fishes sold in Uyo Eastern Nigeria. *World Journal of Agricultural Science* **4, 346-350**. Retrieved from https://www.semantic-scholar.org/paper/Mycofloral-of-Smoke-Dried-Fishes-Sold-in-Uyo-Easte-Adebayo-Tayo-Onilude/2806ca1c6-f224a7ba7ce4181afc26be19efb22cd

Atapattu, R. and Samarajeewa, U. (1990). Fungi associated with dried fish in Sri Lanka. *Mycopathologia* **111, 55-59.**

Alberts, J. F., Engelbrecht, Y., Steyn, P. S., Holzapfel, W. H. and Van, Zyl. W. H. (2006). Biological degradation of Aflatoxin B1 by *Rhodococcus erythropolis* cultures. *International Journal of Food Microbiology* **109, 121-126.**

Bhatnagar, D., K. C. Ehrlich. and T. E. Cleveland. (2003). Molecular genetic analysis and regulation of Aflatoxin biosynthesis. *Applied Microbiology and Biotechnology* **61, 83-93.**

Do, J. H. and Choi, D-K. (2007). Aflatoxins: Detection, toxicity, and biosynthesis. *Biotechnology and Bioprocess Engineering* **12, 583-593.**

Flajs, D. and Peraica, M. (2009). Toxicological properties of citrinin. *Archives of Industrial Hygiene and Toxicology* **60, 457-464.**

Frisvad, J. C., Skouboe, P. and Samson, R. A. (2005). Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B1, sterigmatocystin and 3-O-methylsterigmatocystin, *Aspergillus rambellii* sp. nov. *Systematic and Applied Microbiology* **28, 442-453.**

Geisen, R. (1996). Multiplex polymerase chain reaction for the detection of potential aflatoxin and sterigmatocystin producing fungi. *Systematic and Applied Microbiology* **19, 388-392.**

Hassan, A. A., Hassan, M. A., El Shafei, H. M., El Ahl, R. M. H. S. and El-Dayem, R. H. Abd. (2011). Detection of Aflatoxigenic mould isolated from fish and their products and its public health significance. *Nature and Science* **9, 106-114.**

IARC. (2002) Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Summary of data reported and evaluation. IARC Monographs on the evaluation of the carcinogenic risk to humans. Vol. 82. International Agency for Research on Cancer, Lyon, France.

Jonsyn, F. E. and Lahai, G. P. (1992). Mycotoxigenic flora and mycotoxins in smoke dried fish from Sierra-Leone. *Nahrung (Food)* **36, 485-489.**

Leszczynska, J., Maslowska J., Owczarek A. and Kucharska, U. (2001). Determination of aflatoxin in food products by the ELISA method. *Czech Journal Food Science* **19, 8-12.**

Mohamed, S. (2013). Toxigenic fungi and mycotoxin production in Maldive fish (smoked dried Tuna fish). Thesis of Doctor of Philosophy. Massey University, Palmerston North, New Zealand.

Owaga, E. E., Onyango, C. A. and Njoroge, C. K. (2009). Investigation of mycoflora on dagaa (*Rastrineobola argentea*) as affected by washing and drying methods. *Journal of Applied Biosciences* **19, 1074-1081.**

Pitt, J. I. and Hocking, A. D. (2009). *Aspergillus* and related Teleomorphs. *In:* Fungi and Food Spoliage. 3th Edn. Springer Science + Business Media. New York. **pp. 275-295.**

Prakash, S., Jeyasanta, I., Carol, R. and Patterson, J. (2011). Microbial quality of salted and sundried sea foods of tuticorin dry fish market southeast coast of India. *International Journal of Microbiological Research* **2, 188-195.**

Rachmawati, S., Lee, A., Murdiati, T. B. and Kennedy, I. (2004). Pengembangan *Enzyme Linked Immunosorbent Assay* (ELISA) teknik untuk analisis aflatoksin B1 pada pakan ternak (Development of *Enzyme Linked Immunosorbent Assay* (ELISA) for Aflatoxin B1 analyzed from feed). *In: Proceeding of Parasitology and Veteriner Toxicology Conference*. Pusat Penelitian dan Pengembangan Peternakan. Bogor, Indonesia. **pp. 11-148.**

Rahayu, E. S., Raharjo, S. and Rahmania, A. A. (2003). Aflatoxin contamination during corn production in East Java. *Agritech* **23, 174-183.**

Rahayu, E. S., Sardjono. and Samson, R. A. (2014). Pengenalan Jamur Benang pada Bahan Pangan (Introduction of mold in Food). *In:* Jamur Benang pada Bahan Pangan (Mold in Food). Yogyakarta: PT. Kanisius. **pp 1-17.**

Rahimi, P., Sharifnabi, B. and Bahar, M. (2008). Detection of aflatoxin in *Aspergillus* species isolated from pistachio in Iran. *Journal of Phytopathology* **156, 15-20.**

Rodrigues, P., Soares, C., Kozakiewicz, Z., Paterson, R. R. M., Lima, N. and Venancio, A. (2007). Identification and Characterization of *Aspergillus flavus*

and Aflatoxins. *In:* Communicating Current Research and Educational Topics and Trends in Applied Microbiology. A. Méndez-Vilas (Ed.) Formatex, Spain. **pp. 527-534.**

Samuel, N., Ezri, Y., Farah, R., Igor, V., Hussein, A., Rubinshtein, O. and Assy, N. (2009). Acute aflatoxicosis resulting in fulminant hepatic failure and rhabdomyolysis. *Gastroenterology Research* **2, 48-50.**

Santoso, I., Gandjar, I., Sari, R. D. and Sembiring, N. D. (1999). Xerophilic moulds isolated from salted and unsalted dried fish from traditional markets in Jakarta. *Indonesian Food and Nutrition Progress* **6, 55-58.**

Sharma, R. (2012). Pathogenicity of *Aspergillus Niger* in plants. *Cibtech Journal of Microbiology*, **1, 47-51.**

SNI (Indonesian National Standard) 01-2721-1992. Ikan Asin Kering (Dried Salted Fish).

Sutarni. (2013). Factors affecting production of salted anchovy fish preservation at Labuhan Maringgai subdistrict East Lampung Regency. *Jurnal Ilmiah ESAI* **7(1), 1-14.**

Yu, J., Chang P. K., Ehrlich K. C., Cary J. W., Bhatnagar D., Cleveland T. E., … and Bennett J. W. (2004). Clustered pathway genes in aflatoxin biosynthesis. *Applied and Environmental Microbiolgy* **70, 1253-1262.**

Yu, J. (2012). Current understanding on aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination. *Toxins* **4, 1024-1057.**