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Short Communication

A novel filamentous fungus *Acremonium charticola* isolated from gathot (an Indonesian fermented dried cassava)

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Abstract

Gathot is a traditional fermented dried cassava originated from East and Central Java Indonesia, which is characterized by the black colour of inside and outside part of the tuber. The black colour has been related to fungi growing in the tuber during the fermentation process. The aim of this study was to isolate and identify fungi from gathot produced according to the method applied in Central Java. The peeled cassava tubers were washed and let to stand on the roof (exposed to rainwater and sunshine) for approximately 1 month, until the tubers were dry and black. Direct isolation method was performed when isolating fungi from gathot, in which gathot was ground into flour and placed on potato dextrose agar (PDA) medium. Macroscopic and microscopic identification of fungi was then performed. Based on the mycological characteristics, filamentous fungi *Acremonium charticola* and *Rhizopus oryzae* were the two species of fungi isolated from gathot made in Central Java Indonesia.

Introduction

Fungi are among the most abundant and ubiquitous microorganisms in nature. Fungi constitute a group of living organisms that are devoid of chlorophyll, and thus cannot manufacture their own food (Kim, 2003). As heterotrophic organisms, fungi obtain their carbon and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal), whilst others obtain their nutrients from dead plants or animals (Carris *et al*., 2012). Most fungi associated with plants are saprotrophs, in that fungi break down cellulose, hemicellulose and lignin to obtain their nutrients (Carris *et al*., 2012). In Indonesia, particularly in East and Central Java, this feature has been exploited to produce fungal-fermented cassava product called gathot (Prabawati *et al*., 2011).

It is well known in Indonesia that there is plenty of region-to-region variability in preparing/making one food including gathot. In East Java, to make gathot the sun-half-dried cassava tubers were put in a closed container for 3 days until the tubers were grown by fungi (Purwandari, 2000; Purwandari *et al.*, 2014). In Central Java, gathot is made by exposing the tubers to rainwater and sunshine for approximately 1 month until patchy black area is developed inside the tubers (Prabawati *et al*., 2011). It is generally accepted that the condition of neutral or mildly acidic under pH 7 are required for the optimal growth of fungi (Dix and Webster, 1995). Given that rainwater is slightly acidic (Charlson and Rodhe, 1982), the direct exposure of tubers to rainwater seems to enhance the growth of fungi in the tubers. However, it should be noted that rainwater contains high concentration of sodium and chloride, which is originally derived from sea salt (Cushing *et al*., 2006). Matsuda *et al*. (2006) reported that some species of fungi are less tolerant to higher sodium chloride (NaCl) concentrations than the other species. In this notion, the direct exposure to rainwater during the process of making gathot in Central Java may indirectly determine the species of fungi growing in the cassava tubers. The aim of this present study was to isolate and identify fungi from gathot made in Central Java, Indonesia. It was expected that species of fungi isolated from gathot made in Central Java would be different from that of isolated from gathot made in East Java due to the different production methods. Note that Botryodiplodia theobromae Pat. was a dominant fungus growing inside of gathot made in East Java, as reported by Purwandari (2000).

Materials and Methods

Preparation of gathot

Gathot was made from cassava tubers at their fully ripe stage. The process was initiated by peeling the tubers. The peeled tubers were then washed thoroughly with fresh water and sun-dried. The tubers were let to stand in an open space (on the roof) and exposed to rainwater and sunshine for approximately

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Table 1. Comparison of morphological characteristics of *A. charticola* and *R. oryzae* isolated from gathot with previous description of the respective fungi

Characteristics		Isolate in present study	Previous descriptions‡
A. charticola			
Colony	Colour	Pinkish	Whitish or pink
Conidiophores	Shape	Branched	Often branched
Conidia	Shape	Slimy head, ellipsoidal to short-cylindrical	Slimy head, ellipsoidal to short-cylindrical
	Size	4.4 × 2.0 µm	3.2-4.5 × 1.4-2.0 µm
R. oryzae			
Colony	Colour	Brownish-grey	Whitish becoming brownish-grey with age
Sporangium	Shape	Globose	Globose
	Size	35-184 µm in diameter	50-200 µm in diameter
Sporangiospore	Shape	Sub-globose	Sub-globose
	Size	3-10 µm in length	4-10 µm in length
Sporangiophore	Size	646-858 µm in length	150-2000 µm in length
Columellum	Shape	Globose	Ovoid or globose
	Size	106-140 µm in diameter	30-120 µm in diameter

‡Samson *et al.* (2004)

1 month, until the tubers were grown by black fungus and give patchy greyish appearance in the exterior and interior of tuber. The dried moldy tubers (called gathot) were then collected and stored in a dry space until used. In Central Java, the process of making gathot is done mostly on the rainy season due to the lack of rainfall during the dry season.

Preparation of potato dextrose agar (PDA) medium

The PDA medium was prepared according to Yudiarti *et al.* (2012). In brief, 200 g of peeled and sliced potatoes were boiled in 1 L water until the potatoes became soft. The solution was strained through cheesecloth and adjusted filtrate to 1 L. Prior to autoclaving, dextrose (20 g) and agar powder (17 g) was added to the solution. Chloramphenicol (250 mg) was added to the solution and shaked thoroughly. The 10 mL of the solution was subsequently poured into a petri dish.

Isolation of fungi from gathot

Isolation was conducted based on Yudiarti *et al.* (2012) with some modifications. Prior to isolation, gathot (5 samples) was crushed and ground into flour. The flour was aseptically placed on potato dextrose agar (PDA) medium in sterile petri dishes, and incubated aerobically at 37°C for 1, 2 and 3 days. At these respective days of incubation, the plates were taken out from the incubator and observation (visually) was performed. Each fungus growing on the plate was reseeded into PDA and incubated for 2 days. Purification was always conducted until each plate contained only one fungus.

Identification of isolates

Fungus was identified in macroscopic and microscopic levels according to Yudiarti *et al.* (2012). The macroscopic identification was performed by re-subculturing and observing (naked eye observation) the colonies of fungi growing on PDA. The microscopic identification was performed first by making a microscope slide, i.e., taking the fungus from PDA and placing it on a rectangular piece of glass. The slide was then checked under the light microscope to see the conidiophores, conidia, sporangium, sporangiospore, sporangiophore and columellum.

Results and Discussion

Gathot is a fermented dried cassava that is traditionally produced by local people in East and Central Java Indonesia. In this study, gathot was characterized by the black-pinkish colour due to the fungi growing inside and outside of the tuber during the fermentation process (Figure 1). Note that gathot is made through spontaneous fermentation, and that ubiquitous fungi in nature may involve in the fermentation process. In East Java, Purwandari (2000) have isolated several fungal species from gathot, with dematiaceous fungus Botryodiplodia theobromae Pat. being a dominant fungus found in the tuber. As mentioned earlier, we speculated that the exposure of cassava tubers to rainwater when making gathot in Central Java might determine the species of fungi growing in the tubers. Indeed, two colonies of fungi with different colour, i.e. pinkish (Figure 2) and brownish-grey (Figure 3), were observed after purification of fungi on PDA.

Figure 1. The pieces of gathot made in Central Java, which is characterized by the black-pinkish colour of inside and outside part of the tubers

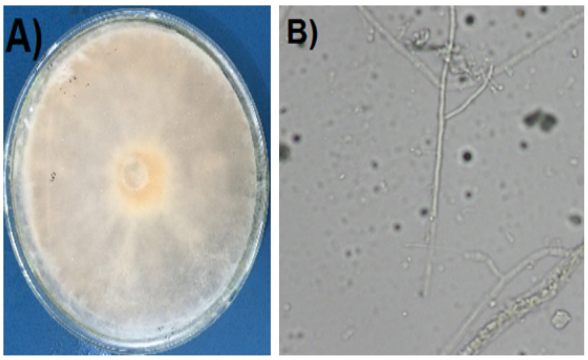

Figure 2. Morphology of *A. charticola* isolated from gathot. A) Colonies of *A. charticola* on PDA after 2 days of incubation (characterized by the pinkish colour). B) Microscopic morphology of *A. charticola*

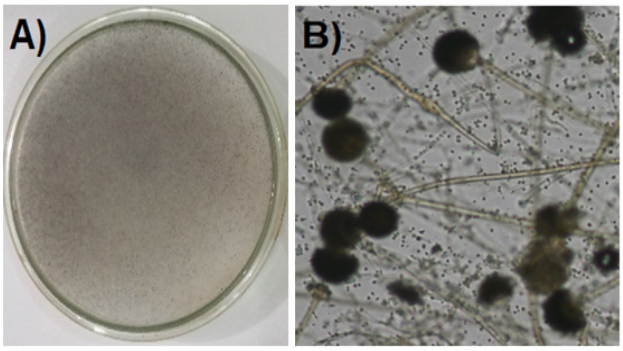

Figure 3. Morphology of *R. oryzae* isolated from gathot. A) Colonies of *R. oryzae* on PDA after 2 days of incubation (characterized by the brownish-grey colour). B) Microscopic morphology of *R. oryzae*

Under the light microscope, it could be seen that the pinkish colony had the branched conidiophores. In addition, this colony had a slimy head, ellipsoidal to short-cylindrical conidia with the size of 4.4 × 2.0 µm. Based on these mycological characteristics (Table 1), the fungus was identified as *A. charticola* (Samson *et al*., 2004). According to the microscopic observation, the brownish-grey colony had the globose sporangium with 35-184 µm in diameter. The sporangiospore was sub-globose with 3-10 µm in length. Sporangiophore of the colony was 646-858 µm in length, while the columellum was globose and 106-140 µm in diameter. From these characteristics (Table 1), the fungal isolate was identified as *R. oryzae* (Samson *et al.*, 2004).

An interesting result was seen in our present work, in which only two species of fungi (mentioned above) grew outside and inside of gathot pieces during the fermentation. Literature studies show that *A. charticola* (Abdel-Lateff *et al*., 2002) and *R. oryzae* (Vala and Sutariya, 2012; Vala, 2014) are salt-tolerant. Thus, the fungi could grow well in the tubers exposed to rainwater, whereas the non-salt-tolerant fungi were probably not able to grow in the tubers. Hence, in this study *A. charticola* and *R. oryzae* were the only two species of fungi isolated from gathot made in Central Java, Indonesia.

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