



## Prevalence of Viable *Monascus* van Tieghem Species in Fermented Red Rice (Hong Qu Mi) at Consumer Level in Selangor, Malaysia

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### ABSTRACT

Red rice is a fermented product consumed widely throughout Asian countries as food colorant, food flavour and food preservative. Fermentation of red rice is accomplished by inoculating individual or several *Monascus* species onto white rice grains during which time the fungal starters biosynthesize the intense red pigments which are subsequently assimilated into the final product. The present work was undertaken to establish the prevalence of viable Monascal fungal starters involved in red rice fermentation from red rice samples obtained at consumer level in Selangor, Malaysia by means of morphological characterization. Macromorphology examined the size and texture of colonies on growth plates, and colony growth rates while micromorphology assessed the size and shape of conidiospores and texture of conidiophores and hyphae. A total of 50 red rice samples were obtained from which isolation and characterization of Monascal fungal starters were performed. From the red rice samples, *Monascus purpureus* Went was found to be the key Monascal fungal starter from which the isolation frequency was 58% as compared to that of *Monascus pilosus* K. Sato which was 42%. No strain of *Monascus ruber* van Tieghem was isolated. Since 100% of red rice samples gave viable spores which germinated at favourable conditions, there is an elevated risk of citrinin contamination of the final product. Therefore, further mycological and toxicological analyses on fermented red rice are warranted to raise public awareness on consumption of red rice.

### INTRODUCTION

Red rice is a product of the solid-state fermentation of white rice by several species of the genus *Monascus* van Tieghem (Ascomycota). The fermented product exhibits a distinctive bright red-purple colouration which is due to the pigments being synthesized by Monascal fungal starters during fermentation. Throughout the Asian countries where the consumption of red rice is widespread, it is known by many names; *Angkak* in Indonesia, *Beni Koji* in Japan, and *Hong Qu Mi* in China [1]. By composition, red rice predominantly contains rice starches and sugars, fungal polyketides, fatty acids, pigments, and condensed tannins [2].

The genus *Monascus* was revised by Hawksworth and Pitt in 1983 due to various similar isolates being assigned to different taxa. Based on physiological and morphological characteristics, three species were recognized: *Monascus pilosus* K. Sato, *Monascus purpureus* Went, and *Monascus ruber* van Tieghem [3]. Long before the genus was revised, these species have already been utilized as fungal starters in the fermentation of red rice because of their pigments-producing ability [4,5]. Despite possessing various health-improving characteristics (mainly attributed to the discovery of monacolin K as anti-hypercholesterolemic) [6], the *Monascus* species also synthesize monascidin A which has subsequently been found to be identical to the mycotoxin citrinin.

The synthesis of citrinin by the *Monascus* species has been investigated and reported [7,8]. Several studies of citrinin toxicity which were conducted on dogs [9], human kidney cells [10], monkey kidney cells [11], rats [12], and mouse embryos [13] described citrinin as teratogen (a substance capable of interfering with foetal development), hepatocarcinogen (a substance capable of inducing liver cancer), and nephrocarcinogen (a substance capable of inducing kidney cancer). However, due to limited conclusive data of citrinin's toxicity on an actual human subject, the International Agency for Research on Cancer categorized citrinin in Group 3 as "not classifiable as to its carcinogenicity to humans" [14].

In Malaysia, the consumption of red rice by the Malaysian Chinese (Malaysians of Chinese descent) especially in the state of Selangor (26.4% Malaysian Chinese) [15], is relatively high and on a daily basis. This is reflected by the operation of numerous traditional Chinese shops within small municipal districts, and by the fact that red rice is sold at a fast turn-over rate. The utilization of red rice varies from direct consumption; as food flavour, food colorant, or food preservative; to the fermentation of traditional Chinese red wine. Hence, there is an elevated probability that consumers would ingest citrinin as a result of the frequent consumption of red rice. The fact that red rice consumption as alternative medication has not been approved by the Food and Drug Administration (FDA), which is mainly due to the presence of varying concentrations of citrinin [16], must be taken into serious consideration.

The objectives of the present work were two-fold; (1) to morphologically characterized the Monascal fungal starters isolated from the red rice and thereby establishing the prevalence of viable *Monascus* spp. in red rice obtained at consumer level in Selangor, Malaysia, and (2) to collect general information and knowledge on red rice consumption pattern, frequency in dietary intake, and the intended use of the red rice by the local Malaysian Chinese in Selangor, Malaysia.

## MATERIALS AND METHODS

### Materials

Malt Extract Agar (MEA; Oxoid, England) for fungal isolation was obtained commercially and prepared according to manufacturer's instruction (50 g MEA powder in 1 L distilled water). The heat-stable anti-bacterial compound chloramphenicol (Oxoid, England) was added (0.01% w/v) to the growth medium to inhibit bacterial contamination. The growth medium was then autoclaved (Meadowrose, England) at 121 °C for 15 minutes at 15 psi. Autoclaved growth medium was left to cool down for 15 minutes before being poured into 90 mm Ø Petri plates (≈15-20 mL per plate) and left to solidify. Solidified Petri plates were placed in sealed polyethylene bags and refrigerated in a cold room at 4 °C for subsequent use.

Red rice samples ( $n = 50$ ; 100 g each) were purchased from traditional Chinese medicine shops throughout the nine administrative districts in Selangor, Malaysia. During sampling, the informal "over-the-counter" approach was employed to collect information on the general red rice consumption pattern and frequency of the local Malaysian Chinese. The samples were collected in sterile bottles, labelled, and taken to the laboratory for immediate examination, or refrigerated at 4 °C until further analysis.

### Isolation of Monascal fungal starters

The isolation of Monascal fungal starters was performed on MEA by the direct-plating technique in which 5 g of each red

rice sample were aseptically spread onto the agar in replicates ( $n = 3$ ). The inoculated agar plates were incubated at ambient temperature ( $27.5 \pm 0.5$  °C) for seven days after which the colonies grown were aseptically sub-cultured onto fresh MEA to obtain axenic (pure) cultures.

### Characterization of *Monascus* spp.

Taxonomic characterization of *Monascus* spp. was done morphologically (macro and micro) on axenic cultures. Macro-morphologically, the colour, texture, and size of *Monascus* spp. colonies, and the colour of the surrounding agar were observed using a dissecting microscope (Olympus, Japan), and the observed characteristics were recorded.

Micro-morphologically, the characteristics of mycelia, hypha, aleurioconidia, cleistothecia, asci, and ascospores were observed using a light microscope (Nikon, Japan) by means of the wet-mound slide technique, and the observed characteristics were recorded. Recorded characteristics were then analyzed and compared, and species were determined according to [3].

## RESULTS AND DISCUSSION

### Consumption of red rice in Selangor

In 2010, 6.4 million out of a population of 26 million Malaysians are Malaysian Chinese from which 1.4 million Malaysian Chinese are residing in Selangor making it the state with greatest number of the ethnic group [15]. The Malaysian Chinese community in Selangor still adhere to customary and traditional practice including the application of Traditional Chinese Medicine (TCM) in daily life.

During the sampling period (January-June 2009) from TCM shops throughout Selangor, it was observed that these shops thrived successfully with several shops operating even within small Malaysian Chinese establishments. Generally, the Malaysian Chinese community consumes red rice similarly to mainland Chinese, Taiwanese, or Korean communities where it is either consumed directly as porridge, or sprinkled on meat or vegetables to impart red colouration, to eliminate meaty odour, and to bring out distinctive aroma and taste. Red rice is also used locally in the fermentation of traditional Chinese red rice wine (*arak samsu*).

The frequent shortage of red rice supplies during the sampling period reflected the high demand of the product among the Malaysian Chinese community. On average, these shops sold 25-50 kg of red rice per month subject to area density (large districts with fewer shops sold more) with consumers buying 0.5-1.0 kg of red rice depending on the application and cooking method. As compared to ordinary white rice (US\$1-2 per kg), red rice is slightly more expensive at US\$5-10 per kg which is due to its preparation and also being an imported commodity. It was also discovered that the majority of shopkeepers (≈75%) acquired their supplies of red rice from abroad (China, Taiwan, Korea, Thailand; large-scale and modern preparation) with relatively small amounts were acquired from local, small-scale and traditional producers.

Even though the estimated consumption of red rice by the Malaysian Chinese community ranged from daily to weekly, the quantity taken may vary depending on the methods of preparation. To date however, there has been no report on citrinin intoxication which has been linked to red rice consumption. Nevertheless, the product has not been approved as dietary supplement by the United States Food and Drug

Administration (USFDA) [17] due to presence of drug (monacolin) and mycotoxin (citrinin).

#### *Monascus* spp. as fungal starters in red rice fermentation

Fig. 1 depicts the cultural characteristics of *M. purpureus* (above, left), *M. pilosus* (above, right) and isolation frequencies of viable *Monascus* starters in red rice fermentation (below) on MEA after seven days of incubation. According to [3], both species exhibit *plane* (flat), and *sparse* (thinly scattered) to moderately *dense* (compact) colonies on MEA. The most apparent differentiating characteristics between the two are the colony diameter (10-20 mm for *M. purpureus*; 30-40 mm for *M. pilosus*), hyphal texture (floccose for *M. purpureus*; deeply floccose for *M. pilosus*), colony colour (deep red for *M. purpureus*; pastel red for *M. pilosus*), and growth rate (*M. purpureus* slower; *M. pilosus* faster).

In the present work, *M. purpureus* colonies on MEA appeared to be small in diameter (8-22 mm), with little aerial growth, white or reddish mycelium initially, and became strongly pigmented in orange or red shades, usually reddish orange as colonies matured, and soluble pigments (diffused into agar) in similar shades were also observed. Colonies of *M. pilosus* on MEA were relatively larger in diameter (25-45 mm), fimbriated (*with edges*), floccose (*cottony*), with pastel red margins, and deep red centres.

*M. purpureus* was present in 29 samples (58%) and *M. pilosus* in 21 samples (42%). Although the difference in both isolation frequencies appeared statistically insignificant, the slightly higher isolation frequency of *M. purpureus* might be attributed to the fact that it biosynthesizes higher concentration of pigments [18] which is the intended use of it as starter culture for fermentation, and hence more favourable as a *Monascus* fungal starter.

However, no *M. ruber* strain was isolated from any of the red rice samples. Although *M. ruber* is relatively the most widespread species of genus *Monascus*, it is infrequently utilized as a *Monascus* fungal starter [3]. Nevertheless, several investigations have recently been performed describing the application of *M. ruber* as a *Monascus* fungal starter [19, 20].

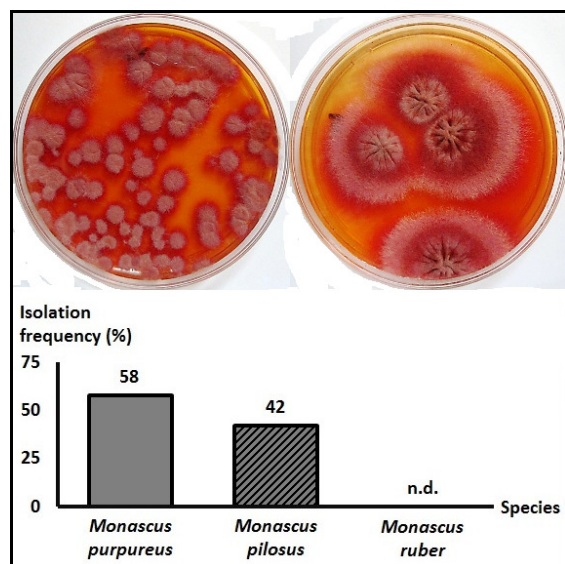


Fig. 1. Plate colonies of *Monascus purpureus* (above, left), *Monascus pilosus* (above, right), and isolation frequencies of *Monascus* spp. on red rice (below). n.d.; not detected.

During the red rice fermentation, two approaches are currently practiced; traditional (small-scale), and modern (large-scale). Apart from production scale, the only other apparent difference between both approaches is the condition in which the red rice is dried. In the traditional approach, the fermented rice is air-dried as opposed to oven-dried as in the modern approach. However, in both approaches, the type of rice is usually similar (ordinary, non-glutinous white rice to avoid clumping and to ease drying), and *Monascus* spp. are the fungal starters. Being the fungal starters, *Monascus* spp. were inoculated into the rice grains to initiate fermentation after which the *Monascus* spores remained affixed to the final product regardless of the different drying conditions of the traditional or modern production. When the condition is favourable, these *Monascus* spores will be highly viable. As red rice holds a pharmacological standing in traditional Chinese medication, it is thus considered as an oral non-liquid herbal preparation and included in the dietary intake only as supplement.

In conclusion, the present work demonstrates that the occurrence of *Monascus* spp. is inevitable in all red rice samples (100%) obtained at consumer level in Selangor, Malaysia. Based on previous researches cited earlier, the utilization of *Monascus* spp. as fungal starters in red rice fermentation will subsequently culminate in citrinin contamination in the final product. It is therefore recommended that further analyses should be performed on actual fungal loads (CFUs/g dry weight) and citrinin concentrations ( $\mu\text{g/g}$  dry weight) on red rice products. Malaysian limits for fungal loads in herbal products (less than  $5.0 \times 10^2$  CFUs/g dry weight) [21], and Malaysian limits for fungal toxins in food products (5  $\mu\text{g/kg}$  or 5 ppb) [22] should serve as regulatory basis in the hazard analyses.

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