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# A Comparative Analysis of the Citrinin Encoded DNA Sequence from A Collection of *Monascus purpureus* Isolates

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**Abstract :** *Monascus purpureus* is fungal species commonly used in the red mold fermented rice production or known as angkak. Angkak is an important source of the natural coloring agent for food as well as bioactive compounds for nutraceuticals. However, *Monascus* may also produce hepato-nephrotoxic citrinin. To some extent the citrinin is genetically regulated by the pksCT gene. Here we present a comparative analysis of pksCT DNA sequences derived from ten isolates of *M. purpureus*. The phylogenetic analysis indicates that the pksCT sequence is closely related with the ones of reference *M. aurantiacus* and *M. ruber*. However, the genetic distance analysis does not reveal the common ancestry yet. More analysis indicates there are a gap and substitution at the position 14698 to 15040. Whether this is a significant mutation need to be further studied since two isolates still showed production of the citrinin as the other *Monascus* isolates possibly have similar potency. Therefore, care must be concerned in the angkak production.
**Keywords :** Monascus purpureus, Comparative analysis, Citrinin Encoded DNA Sequence.

## Introduction

*Monascus* spp have been used in Eastern Asia for making traditional foods for centuries [1, 2, 3]. However, the commonest of microorganism for production of *Monascus* fermented rice (MFR) or angkak is *M. purpureus* [1, 3, 4, 5]. The discovery of monacolin K over twenty years ago, has made MFR or angkak has increasingly become a common functional food for hypolipidemia treatment [6]. It has been known that monacolin K can inhibit the biosynthesis of cholesterol. However, since citrinin [$C_{13}H_{14}O_5$; IUPAC, (3R,4S-trans)-4,6-dihydro-8-hydroxy-3,4,5- trimethyl-6-oxo-3H-2-benzopyrane-7-carboxylic acid] was found as mycotoxin produced by *Penicillium*, *Aspergillus*, and *Monascus* species, the safety of MFR is must be paid to the hepatotoxicity and nephrotoxicity of citrinin. This toxin is suspected to be a carcinogen leading to renal tumors. If citrinin level is at high concentration although its monacolin K concentration is higher, the use of MFR for functional food will unacceptable [5,6].

As both monacolin K and citrinin are known as polyketide derivatives. Therefore, it causes they are unavoidably formed at the same time. Many researches have been made to investigate the culture condition and



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the method for decreasing or removing citrinin formation in addition to retain monacolin K formation in MFR. The citrinin accumulation fundamentally be influenced by pksCT gene expression of the *Monascus* strain, it can be accomplished by PCR of the pksCT gene [6].

There was also indication that it was the effective way to distinguish the citrinin-producing *Monascus* strain by analyzing the pksCT gene with genomic PCR [5, 7].

Natural mutation also can be considered as it is a common happened which can occur during such long-term period. The mutation can be insertion or substitution or delete one or more nucleotides of a gene [8] so it expression will be change such as non-producing toxic substance, citrinin for instance [5, 9].

The objectives of this genetic study were to know mutation occurred at a partial sequence of pksCT gene encoding citrinin polyketide synthase amongst ten local *Monascus* isolates and also compared to *in silico* analysis.

## Materials and Methods

### Monascus purpureus Isolates

Ten selected isolates of *M. purpureus* (MJ, CS, SPS2, KS, CM, HAN, SER, SUR, SU and SSA) were cultured on Malt Extract Agar 2% (Difco) and incubated at 25°C. To purify the fungal culture, the medium, 100 µg/ml chloramphenicol (Sigma) was added.

### DNA extraction and PCR Amplification

Before DNA extraction, the fungi were cultivated in liquid medium (+ chloramphenicol 100 µg/ml) for three days at 30°C. In the course of DNA extraction and purification work, a kit from "Roche" was applied. The procedure from the manufacture was followed in this process. Each DNA extracts from ten samples was amplified by PCR by using a pair of pksCT primer, pksCT F (5-GGAATTCTGCAGCC AGTGTGGCTATTCACC-3') and pksCT R (5'-GGAATTCTGCAGAAGAGTAATGTCCTT AGG-3') [10]. Amplification condition for PCR were initiated by denaturation stage at 95°C, for 10 minutes; then 30 cycles of 94°C, for 30 seconds, annealing at 55°C for 1 minute and extension at 72°C for 1 minute; and single extension at 72°C for 7 minutes [10].

### Gel electrophoresis

To know the quality and quantity of DNA product from PCR amplification, gel electrophoresis was performed. Gel visualization was achieved by UV trans-illuminator.

### Sequencing PCR products

Dye terminator which was used during PCR was added into reaction solution of cycles sequencing and Big Dye Terminator version 3.1 cycle Sequencing Kit (AB Applied Biosystem). All protocols followed from the manufacture. Primer pksCT F (5-GGAATTCTGCAGCCAGT GTGGCTATTCACC-3') and pksCT R (5-GGAATTCTGCAGAAGAGTAATGTCCTTAGG-3) was used to amplify pksCT gene region. The PCR amplification condition was denaturation stage: 95°C, 10 minutes; 30 cycles of 94°C, 30 seconds, annealing at 55°C for 1 minutes and extension 72°C 1 minutes; single extension at 72°C for 7 minutes. Sequencing products analyzed by ABI PRISM 3100 Genetic Analyzer (ABI Applied Biosystem).

### MFR production

Previously, a race of rice (*Oryzae sativa* L.), IR42 was purchased from market in Cibinong, Indonesia. This race of rice was already known as a good rice for *Monascus* growth during its fermentation for angkak production. An amount of 25 g of rice was placed on Petri dish after treated by soaking in water for 8 h. After the excess of water was removed, the rice was then autoclaved for 15 minute, at 121°C at 1 atm. After cooling, the rice was inoculated with a 5 ml of inoculum (10% of rice medium). The inoculum was a two weeks old of *Monascus* inoculum which previously prepared by cultivation on 2% MEA for two weeks at 30 °C. The inoculated rice was incubated for 12 weeks at 30 °C.

**HPLC analysis of citrinin**

Two MFR samples (SER and SSA) were dried at 50 °C for 24 hours. Sample was extracted by dissolving 1.25 g of angkak with 50 ml of ethanol 70% (pH 8.0) and homogenized by using magnetic stirrer for three hours at 15-25˚C, filtered with 0,45 µm filter paper. The 20 µl of extract was injected with column C18 and detector UV-Vis [10].

**Data analysis**

Analysis quality of DNA sequence products was analysed by using FinchTV [11]. Editing DNA sequences used Bioedit program. DNA sequences alignment was carried out by using Clustal X. Each DNA sequences was searched for its significant alignment by using Blast from NCBI [12]. Each DNA sequences was trimmed by using Bioedit program. Clustal X was used to construct phylogenetic tree. NJPlot was used for viewing diagram of phylogenetic tree.

Each DNA sequences was converted to protein by using Expasy [13]. Protein Blast was carried out by using blastp suite (NCBI) to find significant alignment.

## Result and Discussion

PCR Amplification of a partial sequence pksCT gene of ten isolates *M. purpureus* resulted a single DNA fragment with about 400 base pairs in size based on measurement of DNA size marker (Figure 1). The result showed that PCR amplification by a pair of pksCT primer was successful as it indicated by a single DNA band.

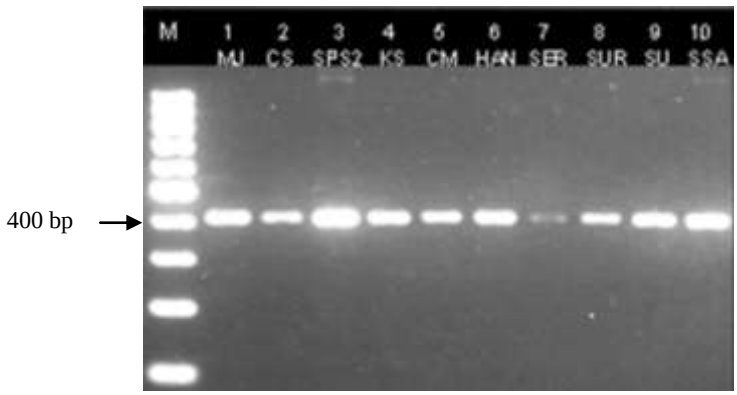


**Figure 1. Photography of gel electrophoresis illuminated by UV to visualize DNA amplification product of ten isolates of *Monascus purpureus* after PCR amplified by a pair of pksCT primer (M=DNA size marker).**

After alignment and trimming of all DNA sequences, the phylogenetic tree was constructed based on DNA sequences of all ten *Monascus* spp. and *M. purpureus* AB167465.1, *M. purpureus* AB243687.1 *M. purpureus* AY954027.1 *M. aurantiacus* EU309474.1, *M. ruber* HQ123042.1, *M. ruber* KT781075.1, *Penicillium expansum* KM603512.1 and *P. expansum* XM_016747316.1 as comparison (All DNA sequences were originated from NCBI database).

The result showed that ten isolates of *M. purpureus* (CM, CS, HAN, KS, MJ, SER, SPS2, SSA, SU, SUR) and *M. purpureus* AB167465.1, *M. purpureus* AB243687.1, *M. purpureus* AY954027.1 were closest related with two other *Monascus* species (*M. aurantiacus* EU309474.1, *M. ruber* HQ123042.1, *M. ruber* KT781075.1) but separated from *Penicillium expansum* KM603512.1 and *P. expansum* XM_016747316.1 which were at another clade (Figure 2).

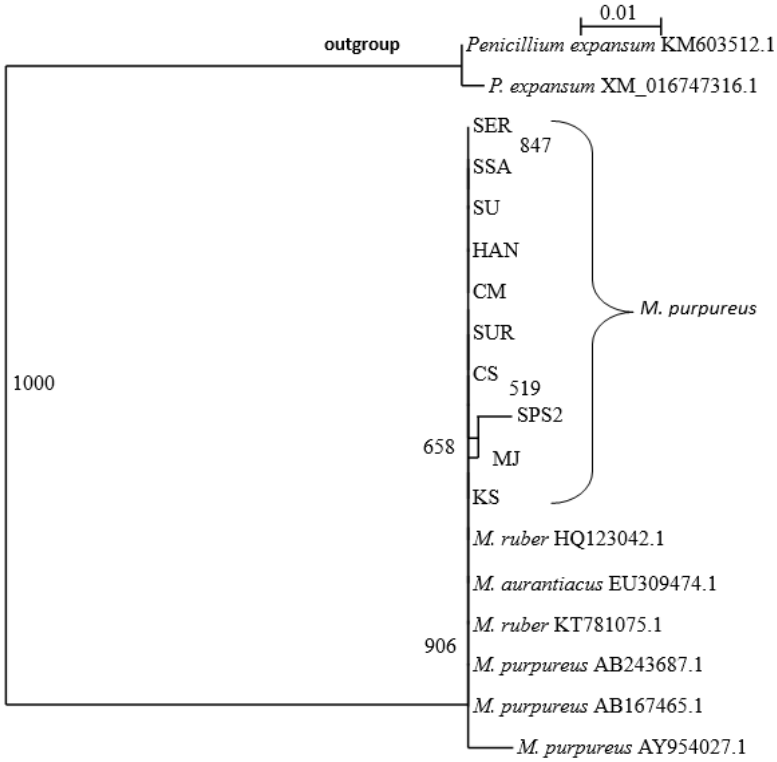


**Figure 2. Phylogenetic tree constructed by Clustal X of a partial sequence of pksCT gene of ten local isolates of *M. purpureus* (CM, CS, HAN, KS, MJ, SER, SPS2, SSA, SU, SUR) and *M. purpureus* AB16, *M. purpureus* AB24, *M. purpureus* AY, *M. aurantiacus* EU, , *M. ruber* HQ, *M. ruber* KT, *Penicillium expansum* KM603512.1 and *P. expansum* XM_016747316.1.**

Based on the phylogenetic tree of their DNA sequences of a partial sequence of pksCT gene, all *Monascus* isolates indicated are nearly identical to each other together with *M. aurantiacus*, *M. purpureus, M. ruber* (Figure 2).

Table 1 showed that five *M. purpureus* isolates (*M. purpureus* CM, *M. purpureus* CS, *M. purpureus* HAN, *M. purpureus* KS, *M. purpureus* SUR) have no mutation of their DNA sequences but other five *M. purpureus* (*M. purpureus* MJ, *M. purpureus* SPS2, *M. purpureus* SU, *M. purpureus* SSA, *M. purpureus* SER) were found having a single or double gap and or substitution of their nucleotide. While *M. purpureus* AY954027.1, *M. purpureus* AB167465.1, *M. purpureus* AB243687.1, *M. aurantiacus* EU309474.1, *Monascus ruber* HQ123042.1, *Monascus ruber* KT781075.1 were found having a single gap, but *M. purpureus* AY954027.1 with double substitution. Two *Penicillium expansum* (KM603512.1 and XM016747316.1) were found having many substitution (*P. expansum* XM016747316.1 with 39 [*P. expansum* KM603512.1 with 38 substitution) but with only a single gap.

We also found conserved region on DNA sequences of a partial sequence of pksCT gene (Table-2). Table-2 showed that from of the ten *M. purpureus* including from of *M. purpureus* AB167465.1 and *M. purpureus* AB243687.1 had only one conserved region with position from14712 to 15041. Two conserved regions (position at 14712 to 14914 and 14916 to 15041) was found when computation included DNA sequences of *M. purpureus* AY954027.1, *M. purpureus* AB167465.1, *M. purpureus* AB243687.1, *M. aurantiacus* EU309474.1, *M. ruber* HQ123042.1, and *M. ruber* KT781075.1 and five conserved regions (Position at 14781 to 14813, 14856 to 14887, 14916 to 14932, 14991 to 15010, 15012 to 15028) after included *P. expansum* KM603512.1 and *P. expansum* XM016747316.1.

This study showed that all ten *M. purpureus* together with *M. purpureus* AB167465.1, *M. purpureus* AB243687.1 except for *M. purpureus* AY954027.1 had a highly conserved region of its pksCT gene but it

showed difference from *M. aurantiacus* EU309474.1, *M. ruber* HQ123042.1, and *M. ruber* KT781075.1 as two conserved regions was determined.

After translation to protein of each DNA sequences of ten isolates of *M. purpureus*, each protein sequence was searched for its alignment in NCBI database by using blastp suite. The result showed that all protein sequences were 100% of its homology with citrinin polyketide synthase of *Monascus ruber* (Table 3).

**Table 1. Mutation of a partial sequence of pksCT gene of *Penicillium expansum* and *Monascus* species isolates which determined at position 14698 to 15040 of its nucleotide sequence.**

| **Species** | **Mutation of Nucleotide Sequence** | |
|---|---|---|
| | **Number/Position of Gap** | **Number/ Position of Substitution** |
| *M. purpureus* HAN | - | - |
| *M. purpureus* SUR | - | - |
| *M. purpureus* CS | - | - |
| *M. purpureus* KS | - | - |
| *M. purpureus* CM | - | - |
| *M. purpureus* MJ | 1/ 150142 | 2/ 150143, 150144 |
| *M. purpureus* SPS2 | 1/ 14711 | - |
| *M. purpureus* SU | 2/ 14708, 14709 | - |
| *M. purpureus* SSA | 2/ 14708, 14709 | - |
| *M. purpureus* SER | 2/ 14708, 14709 | - |
| *M. purpureus* AY954027.1 | 1/ 150142 | 2/ 14701, 14915 |
| *M. purpureus* AB243687.1 | 1/ 150142 | - |
| *M. purpureus* AB167465.1 | 1/ 150142 | - |
| *M. aurantiacus* EU309474.1 | 1/ 150142 | - |
| *Monascus ruber* HQ123042.1 | 1/ 150142 | - |
| *Monascus ruber* KT781075.1 | 1/ 150142 | - |
| *Penicillium expansum* XM016747316.1 | 1/ 150142 | 39/ 14717, 14732, 14741, 14753, 14757, 14768, 14777, 14780, 14814, 14816, 14825, 14828, 14831, 14840, 14846, 14855, 14888, 14897, 14906, 14913, 14933, 14948, 14954, 14957, 14966, 14975, 14979, 14981, 14982, 14983, 14984, 14990, 15011, 15029, 150135, 150136, 150137, 150138, 150141 |
| *P. expansum* KM603512.1 | 1/ 150142 | 38/ 14717, 14732, 14741, 14753, 14757, 14768, 14777, 14780, 14814, 14816, 14825, 14828, 14831, 14840, 14846, 14855, 14888, 14897, 14906, 14913, 14933, 14948, 14954, 14957, 14966, 14975, 14979, 14981, 14982, 14983, 14984, 14990, 15011, 15029, 150135, 150136, 150137, 150138, 150141 |

**Table 2. Conserved regions found after computation of a partial DNA sequence of pksCT gene region by using Bioedit version 7.2.**

| | **Number of conserved regions found** | **Position of Conserved Region** |
|---|---|---|
| Conserved region of DNA sequences of ten *Monascus purpureus* isolates | 1 | Region 1: Position 14712 to 15041 |
| Conserved region of DNA sequences of ten *Monascus purpureus* isolates computed with DNA sequences of | | |
| *M. purpureus* AB167465.1<br>*M. purpureus* AB243687.1 | 1 | Region 1: Position 14712 to 15041 |
| *M. purpureus* AB167465.1<br>*M. purpureus* AB243687.1<br>*Monascus ruber* HQ123042.1,<br>*Monascus ruber* KT781075.1 | 2 | Region 1: Position 14712 to 14914<br>Region 2: Position 14916 to 15041 |
| *M. purpureus* AY954027.1<br>*M. purpureus* AB167465.1<br>*M. purpureus* AB243687.1<br>*M. aurantiacus* EU309474.1<br>*M. ruber* HQ123042.1,<br>*M. ruber* KT781075.1 | 2 | Region 1: Position 14712 to 14914<br>Region 2: Position 14916 to 15041 |
| *M. purpureus* AY954027.1<br>*M. purpureus* AB167465.1<br>*M. purpureus* AB243687.1<br>*M. aurantiacus* EU309474.1<br>*M. ruber* HQ123042.1,<br>*M. ruber* KT781075.1<br>*Penicillium expansum* KM603512.1<br>*P. expansum* XM016747316.1. | 5 | Region 1: Position 14781 to 14813<br>Region 2: Position 14856 to 14887<br>Region 3: Position 14916 to 14932<br>Region 4: Position 14991 to 15010<br>Region 5: Position 15012 to 15028 |

**Table 3. Protein sequences producing significant alignments.**

| ***Monascus purpureus* Isolates** | **Protein Sequence (116 letters)** | **Sequences producing significant alignments** | **Max score** | **Total score** | **Query cover** | **Identity** |
|---|---|---|---|---|---|---|
| CM | LSGECNGALAGGVNVITSPN WYHNLAGASFLSPTGQCKP FDAKGDGYCRGEGVGAVFL KRLSSAIADGDQVFGVIAST KVYQNQNCTAITVPNAISLS ELFTDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 239 | 239 | 98% | 100% |
| CS | RGCNGALAGGVNVITSPNW YHNLAGASFLSPTGQCKPFD AKGDGYCRGEGVGAVFLK RLSSAIADGDQVFGVIASTK VYQNQNCTAITVPNAISLSE LFTDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 231 | 231 | 96% | 100% |
| HAN | LSGECNGALAGGVNVITSPN WYHNLAGASFLSPTGQCKP FDAKGDGYCRGEGVGAVFL KRLSSAIADGDQVFGVIAST | citrinin polyketide synthase [*Monascus* | 239 | 239 | 98% | 100% |

| | | | | | | |
|---|---|---|---|---|---|---|
| | KVYQNQNCTAITVPNAISLS ELFTDVVRQARLEPKDHY | *ruber*] | | | | |
| KS | LSGECNGALAGGVNVITSPN WYHNLAGASFLSPTGQCKP FDAKGDGYCRGEGVGAVFL KRLSSAIADGDQVFGVIAST KVYQNQNCTAITVPNAISLS ELFTDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 239 | 239 | 98% | 100% |
| MJ | LSGECNGALAGGVNVITSPN WYHNLAGASFLSPTGQCKP FDAKGDGYCRGEGVGAVFL KRLSSAIADGDQVFGVIAST KVYQNQNCT | citrinin polyketide synthase [*Monascus ruber*] | 239 | 239 | 98% | 100% |
| SER | SLAGCNGALAGGVNVITSP NWYHNLAGASFLSPTGQCK PFDAKGDGYCRGEGVGAVF LKRLSSAIADGDQVFGVIAS TKVYQNQNCTAITVPNAISL SELFTDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 232 | 232 | 94% | 100% |
| SPS2 | RGCNGALAGGVNVITSPNW YHNLAGASFLSPTGQCKPFD AKGDGYCRGEGVGAVFLKR LSSAIADGDQVFGVIASTKV YQNQNCTAITVPNAISLSELF TDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 231 | 231 | 96% | 100% |
| SSA | SLAGCNGALAGGVNVITSP NWYHNLAGASFLSPTGQCK PFDAKGDGYCRGEGVGAVF LKRLSSAIADGDQVFGVIAS TKVYQNQNCTAITVPNAISL SELFTDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 232 | 232 | 94% | 100% |
| SU | SLAGCNGALAGGVNVITSP NWYHNLAGASFLSPTGQCK PFDAKGDGYCRGEGVGAVF LKRLSSAIADGDQVFGVIAS TKVYQNQNCTAITVPNAISL SELFTDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 232 | 232 | 94% | 100% |
| SUR | LSGECNGALAGGVNVITSPN WYHNLAGASFLSPTGQCKP FDAKGDGYCRGEGVGAVFL KRLSSAIADGDQVFGVIAST KVYQNQNCTAITVPNAISLS ELFTDVVRQARLEPKDHY | citrinin polyketide synthase [*Monascus ruber*] | 239 | 239 | 98% | 100% |

This study revealed that all *Monascus* isolates were genetically potential produced citrinin as translation of a partial sequence of pksCT gene resulted citrinin polyketide synthase which has a role in producing citrinin.

Although this work only used a partial sequence of pksCT gene to study which it was very small segment from the full length of pksCT gene, but this result was important as there was indication of mutation occurred on the gene. A full-length PKS gene (pksCT) has 7,838 bp with a single 56-bp intron. PksCT encodes a 2,593-amino-acid protein that contains putative domains for ketosynthase, acyltransferase, acyl carrier protein (ACP), and a rare methyltransferase. [10] The coding DNA sequence itself is at 1 to 2584. [10] This study use a partial sequence (14698 to 15040) in the area of CDS. Therefore, if mutation occurred in CDS, it could be resulted in change of its gene expression.

This study indicated that all ten local *M. purpureus* and the other *Monascus* species very closed related with mutation indeed occurred although with only single or double changes of its nucleotides so they could be group in a one group but they separated from *Penicillium expansum* although had a similarity at the partial sequence of pksCT gene. This study was also accordance with a review by Srianta et al. [5] BLAST resulted that there was high identity of each PCR product of 14 citrinin-producing *Monascus* strains coming from seven *Monascus* species, and were in accordance with the partial sequences of pksCT gene in *M. purpureus* existing in the Genbank. One conserved region found within *M. purpureus* in this study showed that pksCT gene was more highly conserved compared to *M. purpureus* AY954027.1 *M. aurantiacus* EU309474.1, *M. ruber* HQ123042.1, *M. ruber* KT781075.1.

Interestingly, conserved region in the partial sequence of pksCT gene of all *Monascus* spp. was very different from of *Penicillium expansum* KM603512.1 and *P. expansum* XM_016747316.1. A conserved region of DNA sequence is a conservation of protein-coding sequences leads to produce identical amino acid residues at analogous regions of the protein structure and hence similar function. While conservative mutations change amino acids to analogous chemically residues and so may still not affect the protein's function. Surprisingly, although poorly homologous DNA sequences of pksCT gene as a functional gene to produce citrinin polyketide synthase, the both fungi *Penicillium expansum* and *Monascus* still produce the same toxic substance, citrinin. Both fungi are well known as citrinin producer. With regard to *Penicillium expansum* used in this comparison study, this fungus of any isolates is patulin and citrinin producer [14, 15].

As this study showed a very close on genetic distance amongst *Monascus* species or isolates studied, it was clearly that their ancestry was not yet conclusive.

Although there was mutation on one or two nucleotides of the partial sequence of pksCT gene of *Monascus* species, but the translation came to the same protein, citrinin polyketide synthase of *M. ruber*. Srianta et al [5]. in their review reported that pksCT gene was suggested was a general gene responsible for citrinin biosynthesis in *Monascus* species. Recently, a complete biosynthetic pathway of citrinin has been defined by using gene knockout and heterologous expression strategies of the citrinin gene cluster from *M. ruber* M7 [16].

Although *Penicillium expansum* showed very different conserved region of its pksCT gene as it had many different nucleotides compared to *Monascus* species, in fact its fungus produces citrinin. Therefore, it is suggested that pksCT gene of P. *expansum* species expresses different citrinin product compared to those of *Monascus* species but have same function as toxic substance.

The results showed that two *Monascus* isolates, SER and SSA, showed its production of citrinin (Table-4), by 0.0955 and 0.8724 ppm respectively. These results indicated that these two isolates were regarded as low citrinin producer. These two *Monascus* had mutation by one and two nucleotide gaps. This indicated that although mutation had been occurred but they were still potential in producing citrinin.

**Table 4. Citrinin production of *Monascus purpureus* Isolates.**

| Angkak samples | Citrinin concentration (ppm) | Mean |
|---|---|---|
| *M. purpureus* SER 1 | 0,0517 | 0,0955 |
| *M. purpureus* SER 2 | 0,1393 | |
| *M. purpureus* SSA 1 | 1,0825 | 0,8724 |
| *M. purpureus* SSA 2 | 0,6623 | |

Although the specific legislation for citrinin is limited worldwide, but currently in Japan, the maximum allowed level of citrinin in red fermented rice is 200 ppb. In Taiwan, the regulatory limits of citrinin in red yeast rice (raw material) and *Monascus* products are 5 ppm and 2 ppm, respectively [17]. EFSA, European Food Safety Authority had governed the EU legal limit of 2,000 μg/kg in three of the RYR samples. [18]

The importance of citrinin low-producing isolates or strains for the commercial production of red pigments was indicated after fermentation in experiments to optimize the conditions of fermentation and mediums, the quantity of red pigments reduced with the decrease in citrinin. [7]

Chen et al. [19] reported their inspection the distribution of mycotoxin citrinin biosynthesis genes in *Monascus* strains that the pksCT gene was highly conserved in *M. purpureus*, *M. kaoliang*, and *M. sanguineus*, while the ctnA and orf3 genes were shown to be highly homologous in *M. purpureus* and *M. kaoliang*.

Chen et al. [19] reported that a citrinin-producing phenotype was detected only in *M. purpureus* and *M. kaoliang* using high performance liquid chromatography (HPLC). They also reported the indication on highly conserved citrinin gene cluster in *M. purpureus* and *M. kaoliang* carry out citrinin biosynthesis. Based on the phylogenetic subgroups established with the β-tubulin gene, the citrinin gene cluster can group the species of *Monascus*.

Citrinin has been known to be nephrotoxic, hepatotoxic and carcinogenic to humans and animals. It has been reported that, like ochratoxin A (OTA), citrinin is a likely hazard cause for human Balkan endemic nephropathy (BEN), firstly designated as a chronic tubulointerstitial kidney disease in south-eastern Europe. [20] [21, 22] Recently, the State Food and Drug Administration (SFDA) of China has enforced a limit of citrinin with level of 50 μg/kg in MFR-based functional foods [23].

## Conclusion

Based on a partial sequence of pksCT gene, all *Monascus* species in this study was genetically determined as citrinin producer. They showed highly conserved of their partial sequence of pksCT gene. In fact, citrinin polyketide synthase was found as significant alignment after translation of a partial sequence of pksCT gene which had mutation by one or three nucleotides.

This study revealed that mutation was occurred but citrinin production might still occured. Therefore, the use of every *Monascus* isolates for making *Monascus* red rice must be always in concern as *Monascus* has genetically high potency for producing citrinin during fermentation.

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