Production of γ- Aminobutyric Acid (GABA) by Monascus Purpureus isolated from Angkak, a mold isolated from Angkak in Semarang, Indonesia

E Kusdiyantini, Nurhayati, R S Ferniah

Department of Biology, Faculty of Science and Mathematics, Diponegoro University Semarang 50275

Corresponding author: endangkusdiyantini@lecturer.undip.ac.id

Abstract. Angkak is a rice fermented product that has red colour because of the presence of Monascus sp. This product has long been used as a food colouring and traditional medicine, especially in Asian countries including Indonesia. Angkak is also commercialized in Semarang and used as a medicine for dengue fever. Monascus purpureus is a common mold obtained from Angkak marketed in Semarang. In addition to producing pigments for food, Monascus sp. can synthesize lovastatin or monacolin-K which could reduce blood cholesterol levels also produce γ-aminobutyric acid (GABA) which has physiological functions, such as neurotransmitters. This study aimed to determine the production of GABA produced by Monascus purpureus isolated from commercial Angkak in Semarang. M. purpureus was grown in broth medium (Potato Dextrose Broth) for 14 days in 28°C, and regularly measured the pigment concentration and GABA content every 6 days. The mold was also cultivated in rice solid medium (IR42) for 30 days to measure GABA concentration. The result showed that extracellular pigments were yellow and red with concentration for about 37,358 U/g and 2.6545 U/g, respectively. While intracellular pigment stored in mycelia, the yellow pigment had the highest concentration (30.176 U/g) followed by yellow pigment (7.1475 U/g). GABA content obtained from a broth culture of M. purpureus, Angkak from M. purpureus and commercial Angkak were 0.0796 mg/mL, 0.0332 mg/mL and 0.0203 mg/mL, respectively.

1. Introduction
Monascus sp. is a mould belonging to the family of Monascaceae in Ascomycota phylum. Monascus has more than 20 internationally recognized species, including M. pilosus; M. ruber; M. purpureus; M. floridanus; M. eremophilus; M. pollens; M. sanguineus; M. lunispora; and M. Argentinensis. Monascus mushrooms in Asia have long been used as colouring and food flavourings since their red pigments are safe to use as food colouring in the industry. Monascus pigments have been widely used in South China, Japan, and various countries in Southeast Asia, especially to make red rice wine, red soybean cheese, and anka (brown rice). Monascus pigments generally contain 6 major azaphilone pigments, including yellow pigments: monascin (C21H26O5), ankaflavin (C23H30O3); orange pigment: monascorubrin (C23H26O5), rubropunctatin (C21H22O5); and red pigment: monascorubramine (C22H27NO4), rubropuntamine (C21H25O4). Monascus is also known to produce mycotoxin citrinine such as monascopiridine which is good as a toxic metabolite [1].

The natural red pigment is the result of secondary metabolites from Monascus mould which begin to form in the slow growth phase and increase in the stationary growth phase [2]. The Monascus
pigment is derived from a group of fungal metabolites called azaphilon, which is synthesized from chromophores polyketides and β keto acids through an esterification process. Monascus pigments are stable in the pH range from 2-10. Monascus is resistant to heat and can be autoclaved. Monascus has low water solubility and pigment colours can fade if exposed to light [3]. Monascus pigments according to Timothy (2004) are divided into two, namely intracellular pigments (water insoluble), and extracellular pigments (water soluble).

In addition to producing pigments, Angkak is believed to be a traditional medicine that can reduce blood cholesterol levels because it produces lovastatin compounds. Monascus is also known to produce other secondary metabolites, namely γ-aminobutyric acid (GABA, CH₃CH₂CH(NH₂)COOH) which has an important role in neurotransmitters in the central nervous system [4]. As a supplement, γ-aminobutyric acid (GABA) is sold as a neurotransmitter drug. This compound is a non-essential amino acid that helps maintain brain function so that it remains normal by helping to block impulse related to stress from reaching receptors in the central nervous system. GABA can be produced by plants such as grains [5] and lactic acid bacteria [6], also Monascus sp. [7, 8]. Given the importance of this compound in the world of health, the natural source of production of GABA is still being developed in research to obtain natural resources as a substitute for chemical synthesis.

This study aims to determine the production potential of GABA by Monascus purpureus, moulds isolated from Angkak commercialized in Semarang, Central Java, Indonesia.

2. Method
The study was conducted UPT Laboratory, Diponegoro University, Semarang Indonesia. Materials used in this current study were: Monascus sp. isolates (collection of Biotechnology laboratory of Biology Department, Faculty of Science and Mathematics Diponegoro University); media Potato Dextrose Agar (PDA); media Potato Dextrose Broth (PDB) and rice IR42.

2.1. Preparation of Monascus sp.
Monascus mould was grown on a PDA slant. The culture was then incubated at room temperature for 14 days, then stored at 4°C for further study.

2.2. Inoculum and starter preparation
The spore suspension is made by transferring 3 mL of sterile distilled water into a slanted culture in a test tube. The culture surface to be slanted is eroded so that the spore suspension is obtained. The number of spores was calculated using haemocytometer and spore suspension for stater with a density of 10⁷ spores/ml. The calculation uses the following formula,

\[ \text{Total spore: } E \times 50 \times F \times 1000/\text{ml} \]

\[ E = \text{the total of spore in the box} \]

\[ F = \text{dilution factors} \]

2.3. Monascus growth pattern in broth media
10% (b/v) of spore with a density of 10⁷ spores/mL was inoculated into 250 mL Erlenmeyer which contains 100 ml of Potato Dextrose Broth (PDB) media. Incubation was carried out for 14 days in shakers with 100 rpm agitation in 28°C. Every 6 days samples are taken to measure the concentration of pigments and γ-aminobutyric acid.

2.4. Angkak production by Monascus purpureus using rice solid medium
IR42 rice is washed thoroughly and then soaked with enough distilled water (1:1) for 8-12 hours. The soaked rice is then drained until the water content is reduced. Rice is weighed about 25 grams and put into Petri or a culture bottle for aeration. Sterilization was carried out using autoclave at 121°C for 15 minutes, then cooled to approximately 36°C or dry and not
too hot. Rice as a substrate that is too soft will affect the quality of Angkak. According to [9] with modifications, that inoculation was carried out by adding 2 mL of the ascosporous suspension obtained from 14 days old culture from PDB media. Inoculated rice is then incubated at room temperature for 30 days. The formation of red pigments in rice is known as Angkak. Angkak is then harvested and dried at 40°C for 48 hours or until it is dried using an oven.

2.5. Production of γ-aminobutyric acid (GABA)

a. Production of GABA in broth culture

*Monascus* culture was taken as a sample based on a predetermined incubation time interval. Then centrifuged to obtain a supernatant and then measured its absorbance in spectrophotometry with \( \lambda = 210 \) nm. Calculation of GABA concentrations produced based on standard curves made using pregabalin.

b. Production of GABA in Angkak

The harvested Angkak is weighed as much as 3 g, then added 30 ml of methanol, cornered at a shaker at 40 rpm for 24 hours. The resulting pigment is then filtered with 41 grade Whatman paper, the resulting filtrate is then measured to determine its spectrum by spectrophotometry at \( \lambda \) between 200-600 nm.

3. Results and discussion

3.1. Production of γ-aminobutyric acid on *M. purpureus*

The production of γ-aminobutyric acid in this mould and standard pregabalin 75 mg is measured at wavelengths using between 200-600 nm. This wavelengths is used to get maximum standard spectrum of pregabalin for getting optimum sensitivity in measuring the sample. Spectral results show a maximum at wavelengths between 289 - 301 nm. The absorbance of pregabalin 2,253 (Figure 1), for *M. purpureus* 301 culture and for the value is 292.

![Spectrum of γ-aminobutyric acid (GABA) in pregabalin.](image)

**Figure 1.** Spectrum of γ-aminobutyric acid (GABA) in pregabalin.
Calculation of the concentration of $\gamma$-aminobutyric acid requires a standard curve from pregabalin (Figure 4). Pregabalin (n ((S)-3-aminomethyl-5-methyl hexanoic acid) is a medicine where its chemical structure is analog with GABA, a neurotransmitter inhibitor. Making a standard curve is intended to determine a linear regression equation so that it can be used in the search for a measured absorbant content. This linear regression equation is the relationship between the pregabalin concentration and its absorbance. Figure 4 showed that the intercept value (a) was 0.2858 and the slope value (b) was 33.673 with a correlation value of 0.9862. The correlation value was close to number 1 so that this linear equation was good to be uses as a sample measurement.
[10] have reacted the pregabalin with p-dimethylanobenzaldehyde (pDMAB) as a chromogenic agent to give a light yellow colour (λ_max at 420 nm) for the determination of pregabalin and pregabalin shows a λ_max at 180 nm. While [11] shows that the λ_max at 196.2 nm when pregabalin was reacted with ninhydrin by heating at a temperature of 70-75°C for 20 minutes.

The concentration of γ-aminobutyric acid produced by Monascus purpureus was 0.0796 mg/mL, followed by Angkak made from Monascus purpureus. Commercial Angkak showed the lowest yield in GABA (Table 1). The results were not optimal yet compared with the results of others research.

**Table 1. Production of γ-aminobutyric acid (GABA) in three samples.**

| Sample                        | GABA concentration (mg/mL) |
|-------------------------------|-----------------------------|
| *M. purpureus* in broth culture | 0.0796                      |
| Angkak produced by *M. purpureus* | 0.0332                      |
| Commercial Angkak             | 0.0203                      |

The major factors affecting the production of GABA by microorganisms are temperature, pH, fermentation time and different media additives [12]. The addition of MSG in media give significantly the GABA concentration [13]. The result have also shows the maximum spectrum (λ_max) of pigment at 500 nm (Figure 5). The spectrum shows that λ_max for red pigment in Monascus purpureus that is rubropunctamin, C_{21}H_{26}NO_{4}, and monascrubramin, C_{23}H_{27}NO_{4} [14].

![Figure 5. Pigment spectrum of Monascus purpureus](image)

4. Conclusion

The results showed that Monascus purpureus isolated from Angkak in Semarang had the potential to produce γ-aminobutyric acid (GABA). This research still needs confirmation for concentration and increase production with other methods

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