Use of Mung Bean Sprout (Tauge) as Alternative Fungal Growth Medium

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Abstract. Growth media are essential in mycological studies. Currently, laboratories are using ready-to-use media which are commercially available. However, the prices of such media are high and are a burden on low-funded laboratories. Mung bean sprout (tauge) has been used as a cheap alternative growth medium since 1974, but study comparing performance of the medium with its commercial counterparts has not been reported. This study was done to compare the performances of tauge extract with commercial Potato Extract and Yeast Extract for growing yeast and filamentous fungi. We also endeavoured to optimise the composition of the alternative media by statistical analysis. The results show that the tauge extract medium gave a significantly higher growth rate of *Saccharomyces cerevisiae* and a significantly lower growth rate of *Kluyveromyces marxianus* compared to the commercial media. On the other hand, the growth rates of *Aspergillus oryzae* and *Trichoderma viride* on all media are not significantly different. The optimum composition of tauge extract media for *S. cerevisiae* are 9.6 and 6.8 % (w/v) of tauge and sugar, respectively. For *A. oryzae*, maximum growth is predicted when the medium contains 11.2 and 7.4 % (w/v) of tauge and sugar, respectively.

Keywords: alternative medium, fungal growth, mung bean sprout, tauge extract

1. Introduction
Growing and maintaining fungi in the laboratory using suitable growth media is an essential step in mycological study. Most fungi can grow in media containing natural components, such as potato extract, malt extract, and corn- or oatmeal [1]. The plant extracts provide nutrients for cultivation; they contain amino acids, low molecular weight peptides, and high amounts of carbohydrates [2]. Glucose is frequently added to the mixture to act as a carbon source for fungi [1]. Fungal growth media are commercially available in ready-to-use powder form [2], and many laboratories in developed countries regard them as their standard. Commercial media are relatively expensive, e.g. the price of 500g of Potato Dextrose Agar (PDA) is $114; while 500 gr of Malt Extract Agar (MEA) is $110 [3]. The high medium cost will increase research expenses and become a burden for low-funded laboratories. Hence, cheap alternative media are needed.

Studies have been done to search alternative fungal growth media. Adesemoye & Adedire [4] compared the growth of eight species of fungi (*Aspergillus niger*, *Fusarium moniliforme*, *Penicillium* sp., *Cercospora* sp., *Curvularia pallescens*, *Botryodiplotypodia* sp., *Rhizopus* sp. and *Rhodotorula rubra*) on three media made of cereals (corn, sorghum, and millet). The authors found that the fungal growth was similar to the growth on commercial PDA. Other study done by Ravimannan et al. [5] showed that alternative media made of legumes (cowpea, mung bean, black gram, and soybean) were able to support the growth of *Aspergillus*, *Trichoderma*, *Penicillium*, *Fusarium* and *Sclerotium*, and comparable to PDA. Those studies proved that legumes and cereals are promising as alternative fungal growth media; moreover, they are easily found worldwide as cultivated crops [6].

Mung bean (*Vigna radiata* (L.) R. Wilczek), also known as green gram, is a common crop cultivated in Southeast Asia [6]. In Indonesia, mung bean is popular as a cheap nutritious food and consumed either as seed or as sprouted seed. The sprout, or *tauge* in Indonesian (Figure 1), is rich in protein and...
carbohydrate, approximately 30 and 60% of dry weight, respectively [7,8]. The protein is comprised mainly of phenylalanine, tyrosine, lysine, and leucine [8]; while the carbohydrate mainly is starch (approx. 83%) with a little amount of reducing sugars (approx. 5%) [7].

Figure 1. Mung bean sprout (tauge)

Beside highly nutritious, tauge is also very cheap; the price for 1 kg of the sprout is $1.84 in the public market. Hence, laboratories in Indonesia have used tauge as alternative medium for growing microalgae [9–11] and fungi [12]. However, there is no publication to authors’ knowledge comparing fungal growth on a tauge medium with growth on commercial media. This study was conducted to evaluate performance of tauge as a fungal growth medium compared to potato dextrose and malt extract media. Optimisation of the sprout medium preparation was also studied.

2. Material and Methods

2.1. Material
Mung bean sprouts (tauge) and table sugar (PT. Sugar Group Companies, Jakarta) were obtained from a local market in Yogyakarta, Indonesia. Potato Dextrose Broth (HiMedia), Malt Extract Broth (HiMedia), and Bacteriological Agar (HiMedia) were purchased from LabSatu (Jakarta, Indonesia). Strains of yeast (Saccharomyces cerevisiae FNCC 3012, Kluyveromyces marxianus FNCC 3026) and filamentous fungi (Aspergillus oryzae FNCC 6004, Trichoderma viride FNCC 6013) were obtained from the Centre of Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia.

2.2. Media preparation
Potato dextrose broth (PDB) and malt extract broth (MEB) were prepared according to instructions written on the packages. Bacteriological agar (1.5% w/v) was added to the instructed recipe to prepare solid media (Potato Dextrose Agar, PDA; and Malt Extract Agar, MEA). Tauge extract was prepared by boiling 10% (w/v) of tauge in distilled water for 1.5 hours [12]. The extract was then filtered and restored to its original volume by adding distilled water. Broth medium (Tauge Extract Broth; TEB) was prepared by adding 6% (w/v) of table sugar to the extract, while solid media (Tauge Extract Agar; TEA) was prepared by adding additional 1.5% bacteriological agar to the broth medium. Sterilisation of all media was done in an autoclave (All American 75X, Wisconsin Aluminium Foundry Co. Inc., Manitowoc) at 121 °C for 20 minutes.

2.3. Media preparation
Yeast growth profiles were obtained by growing the strains in TEB, PDB, and MEB with the following method. Fifty millilitres of each medium was placed in 100 ml Erlenmeyer flasks; these flasks were then inoculated with 1 ml of suspension containing approximately 3 x 10^8 cells of S. cerevisiae or K.
marxianus which had been grown for 24 hours in respective media. The flasks incubated in a reciprocal shaking water bath (WNB 14, Memmert GmbH + Co. KG, Schwabach) at 30 °C and 130 strokes per minute for 32 hours. Every 4 hours, 1 ml of sample was withdrawn from the flasks and cell optical density was measured using a spectrophotometer (GeneSys 20, ThermoFisher Scientific, Waltham) at λ 595. The absorbance value was plotted against the growth time, and the specific growth rate (µ) was determined. All experiments were done in triplicate.

Growth profiles of filamentous fungi were obtained by growing A. oryzae and T. viride on TEA, PDA, and MEA in Petri dishes. Each Petri dish was inoculated by the fungus spore using point inoculation and incubated for 153 hours at 30 °C in an incubator (Tv 15b, Memmert GmbH + Co. KG, Schwabach) for A. oryzae; and at room temperature on a lab bench for T. viride. Every 12 hours the colony diameter was measured using a Vernier calliper (Tricle Brand, Shanghai). Colony area was calculated from its diameter, plotted against time, and specific growth rate (µ) was determined. All experiments were done in triplicate.

2.4. Optimisation of tauge medium preparation
A five-level, two-factor central composite design (CCD) was used to determine the effect of various concentrations of tauge and sugar in the sprout medium on the fungal specific growth. The experimental design can be seen in Table 1. Tauge extracts were prepared by boiling various concentrations of tauge and adding various concentrations of sugar according to Table 1. S. cerevisiae and A. oryzae were grown on concentration-varied TEB and TEA, respectively, using methods described in the fungal growth section above. The specific growth rates (µ) of both fungi from each run were calculated as the design responses, and then analysed. All experiments were done in duplicate.

Table 1. Design of experiments used in optimisation of tauge medium preparation

| Run number | Tauge Coded | Tauge Value (% w/v) | Sugar Coded | Sugar Value (% w/v) |
|------------|-------------|---------------------|-------------|---------------------|
| 1          | 0           | 10.0                | 1.414       | 8.8                 |
| 2          | 1           | 15.0                | -1          | 4.0                 |
| 3          | -1.414      | 2.9                 | 0           | 6.0                 |
| 4          | 0           | 10.0                | 0           | 6.0                 |
| 5          | 0           | 10.0                | 0           | 6.0                 |
| 6          | 0           | 10.0                | 0           | 6.0                 |
| 7          | 0           | 10.0                | -1.414      | 3.2                 |
| 8          | 0           | 10.0                | 0           | 6.0                 |
| 9          | 0           | 10.0                | 0           | 6.0                 |
| 10         | 1           | 15.0                | 1           | 8.0                 |
| 11         | -1          | 5.0                 | -1          | 4.0                 |
| 12         | 1.414       | 17.1                | 0           | 6.0                 |
| 13         | -1          | 5.0                 | 1           | 8.0                 |

2.5. Specific growth rate determination and statistical analyses
The specific growth rate (µ) was determined by plotting a natural log of the cell density or the colony area against time; the slope (gradient) of straight line in exponential phase of the plot equal to µ [13]. A two-sample T-test was used to compare the µ of each fungus in the different media. All statistical and central composite design analyses were done in Minitab® 17 (Minitab Inc., State College, Pennsylvania). Fungal growth plots and curves were done in OriginPro® 8.6 (OriginLab Co., Northampton).
3. Results and Discussion

3.1. Fungal growth on different media

The cell densities of the yeasts were plotted against time, and the plots then fitted using the logistic function in OriginPro® 8.6 to obtain their growth profiles (Figure 2). Growth of \textit{S. cerevisiae} on TEB showed a longer lag phase compared to growth on MEB and PDB; the yeast needed 8 hours before starting its exponential phase on TEB, while it only needed 4 hours on MEB and PDB. The longer lag phase indicated that the yeast needed more time to adapt with sucrose as its carbon source in TEB. Unlike glucose which is used directly, \textit{S. cerevisiae} must hydrolyse sucrose into glucose and fructose prior to the intake of the monosaccharides. The hydrolysis is catalysed by invertase, whose production involves a complex transcriptional regulation of the \textit{SUC2} gene [14]. This may well have caused the lag that we observed in our study.

Growth profiles of \textit{K. marxianus} showed similar lag phase times (4 hours). However, the yeast growth on MEB did not produce as many cells as the growth on TEB and PDB; the absorbance of the cells grown on MEB upon reaching the stationary phase was only half the growth on the other two media. Inability of \textit{K. marxianus} to grow on a medium containing maltose as the sole carbon source has been widely known [15]; however MEB, in addition to containing a high amount of maltose (approx. 33% w/v), contains hexose (glucose and fructose) in sufficient amount (approx. 25% w/v) [16] to sustain the yeast growth to some extent.

![Growth profiles of \textit{S. cerevisiae} FNCC 3012 and \textit{K. marxianus} FNCC 3026 in Tauge Extract Broth (TEB), Malt Extract Broth (MEB), and Potato Dextrose Broth (PDB). Line: Growth curve fit using logistic function.](image)

Growth rate of \textit{S. cerevisiae} on TEB was the highest (0.44 ± 0.02 h\(^{-1}\)); on MEB it was 0.38 ± 0.01 h\(^{-1}\), and on PDB it was the lowest (0.37 ± 0.02 h\(^{-1}\)). \textit{K. marxianus} gave the highest growth rate on MEB (0.44 ± 0.01 h\(^{-1}\)); on PDB it was 0.34 ± 0.01 h\(^{-1}\); and it was the lowest on TEB (0.25 ± 0.03 h\(^{-1}\)) (Table 2). TEB contains a high amount of table sugar (6% w/v), which is mainly composed of sucrose; the disaccharide was conveniently utilised by \textit{S. cerevisiae} using invertase enzyme which is known to be produced by the yeast [14,17]. On the other hand, \textit{K. marxianus} showed a lowest growth rate in TEB.
Fonseca et al. [18] reported that the *K. marxianus* growth rate in a sucrose medium was 0.42 – 0.43 h\(^{-1}\). The lower value observed in our study suggests that other factor(s) in a *tauge* medium, beside the carbon source, affect yeast growth. Further study must be taken to investigate it.

**Table 2.** Growth rates of *S. cerevisiae* FNCC 3012 and *K. marxianus* FNCC 3026 in *Tauge* Extract Broth (TEB), Malt Extract Broth (MEB), and Potato Dextrose Broth (PDB). Results with a same superscript letter are significantly different at \(\alpha = 0.05\).

| Strain                  | Growth rates on medium (h\(^{-1}\)) |
|-------------------------|-------------------------------------|
|                         | *S. cerevisiae* FNCC 3012         | *K. marxianus* FNCC 3026 |
| TEB                     | 0.44 ± 0.02\(^{a,b}\)             | 0.25 ± 0.03\(^{c,d}\)   |
| MEB                     | 0.38 ± 0.01\(^a\)                 | 0.44 ± 0.01\(^c\)       |
| PDB                     | 0.37 ± 0.02\(^b\)                 | 0.34 ± 0.01\(^d\)       |

Growth profiles of filamentous fungi were made by plotting the colony areas against time prior to curve-fitting using polynomial function in OriginPro® 8.6 (Figure 3). According to the results, both fungi were in their exponential phases on all media at the end of observation period. *A. oryzae* showed the highest colony area after 144 hours growth on TEA, probably due to the high sugar content in the medium with additional 6% (w/v) sugar beside the ones already contained in the *tauge* extract. *T. viride*, on the other hand, showed similar growth profiles on all media. The results suggest that TEA is a compatible medium for both filamentous fungi; however, the lower colony area produced by *T. viride* compared to *A. oryzae* shows growth speed differences between the respective fungal species. According to Kumhar et al. [19], PDA was proved to be the best medium to grow *T. viride* among commercial media.

**Figure 3.** Growth profiles of *A. oryzae* FNCC 6004 and *T. viride* FNCC 6013 on *Tauge* Extract Agar (TEA), Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA). Line: Polynomial curve fit.
The growth rate of *A. oryzae* on TEA was 0.051 ± 0.002 h⁻¹; on MEA it was 0.050 ± 0.005 h⁻¹; and it was the lowest on PDA (0.047 ± 0.003 h⁻¹). Growth rates of *T. viride* were similar on TEA, MEA, and PDA (0.045 ± 0.001, 0.046 ± 0.002, and 0.046 ± 0.001 h⁻¹, respectively) (Table 3). There are no significant differences between the growth rates; this supports our hypothesis that TEA is a suitable growth media for filamentous fungi.

**Table 3.** Growth rates of *A. oryzae* FNCC 6004 and *T. viride* FNCC 6013 on Tauge Extract Agar (TEA), Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA). Results with a same superscript letter are not significantly different at α = 0.05.

| Strain          | *A. oryzae* FNCC 6004 | *T. viride* FNCC 6013 |
|-----------------|-----------------------|-----------------------|
| Growth rate on medium (h⁻¹) |                      |                      |
| TEA             | 0.051 ± 0.002<sup>ab</sup> | 0.045 ± 0.001<sup>cd</sup> |
| MEA             | 0.050 ± 0.005<sup>a</sup> | 0.046 ± 0.002<sup>c</sup> |
| PDA             | 0.047 ± 0.003<sup>b</sup> | 0.046 ± 0.001<sup>d</sup> |

3.2. *Optimisation of tauge medium preparation*

Effects of *tauge* and sugar concentrations in TEA on *S. cerevisiae* and *A. oryzae* growth rates were investigated using Central Composite Design. The fungal growth responses to each combination in the design can be seen in Table 4. The response surface regressions, contour plots, and response optimizers were calculated using Minitab® 17.1.0.

**Table 4.** Growth rates of *S. cerevisiae* FNCC 3012 and *A. oryzae* FNCC 6004 as a result of *tauge* extract composition optimisation using Central Composite Design.

| Run | Tauge (% w/v) | Sugar (% w/v) | Growth rate (*S. cerevisiae* FNCC 3012) | Growth rate (*A. oryzae* FNCC 6004) |
|-----|---------------|---------------|----------------------------------------|-------------------------------------|
| 1   | 10.0          | 8.8           | 0.3639                                 | 0.0739                              |
| 2   | 15.0          | 4.0           | 0.3374                                 | 0.0653                              |
| 3   | 2.9           | 6.0           | 0.3440                                 | 0.0644                              |
| 4   | 10.0          | 6.0           | 0.3808                                 | 0.0765                              |
| 5   | 10.0          | 6.0           | 0.3705                                 | 0.0709                              |
| 6   | 10.0          | 6.0           | 0.3625                                 | 0.0742                              |
| 7   | 10.0          | 3.2           | 0.3661                                 | 0.0726                              |
| 8   | 10.0          | 6.0           | 0.3821                                 | 0.0749                              |
| 9   | 10.0          | 6.0           | 0.3701                                 | 0.0737                              |
| 10  | 15.0          | 8.0           | 0.3612                                 | 0.0710                              |
| 11  | 5.0           | 4.0           | 0.3605                                 | 0.0694                              |
| 12  | 17.1          | 6.0           | 0.3353                                 | 0.0682                              |
| 13  | 5.0           | 8.0           | 0.3642                                 | 0.0669                              |

Growth rate of *S. cerevisiae* gave a regression model with an acceptable $R^2$ of 83.07%. The contour plot from the model is depicted in Figure 4A. The response optimizer showed that the maximum growth rate of the yeast can be achieved when 9.6 and 6.8 % (w/v) of *tauge* and sugar, respectively, are used. A regression model of the *A. oryzae* growth rate also gave an acceptable $R^2$ of 85.40%. The contour plot of the model can be seen in Figure 4B. The maximum growth rate is predicted to be achieved when the medium contains 11.2 and 7.4 % (w/v) of *tauge* and sugar, respectively.
Figure 4. Contour plot of growth rates ($h^{-1}$) of *S. cerevisiae* FNCC 3012 (A) and *A. oryzae* FNCC 6004 (B) against sugar and *tauge* concentrations.

The initial values of the component concentration for the *tauge* medium (10% (w/v) *tauge* and 6% (w/v) sugar) were taken from a study done by Saono et al. [12]. The authors used the medium to grow mostly yeasts from the genus *Saccharomyces* and *Candida* isolated from Indonesian fermented foods. We suspected the authors already optimized the medium for yeast growth, as our results showed optimum concentration values for *S. cerevisiae* similar to the initial ones. However, optimum concentrations for *A. oryzae* growth were slightly higher than initial values. This can be caused by the higher nutrient requirements of the filamentous fungi to sustain its growth compared to yeasts.

Even though the *tauge* medium is much cheaper than commercial media, lower amounts of sugar used in the medium are preferred. The contour plots (Figure 4) show that lower sugar concentrations can be used to achieve similar growth rates as the optimum ones; the sugar concentration for *S. cerevisiae* growth can be minimized to approx. 4.5% (w/v), and that of *A. oryzae* to approx. 6% (w/v). This can reduce the *tauge* medium cost further and increase its attractiveness as an alternative medium.

4. Conclusions
The *tauge* medium was able to give a similar growth performance of various fungi compared to commercial media. The optimum concentrations of the medium components for yeast growth were 9.6 and 6.8 % (w/v) of *tauge* and sugar, respectively; while for filamentous fungi they were 11.2 and 7.4 % (w/v) of *tauge* and sugar, respectively. Lower concentrations of sugar can be used to some extent to achieve the same growth rate as the optimum concentration.

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