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Utilisation of Local Crops as Alternative Media for Fungal Growth

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ABSTRACT

Potato Dextrose is the most commonly used media for the culturing of fungi. In this study, local crops were used as a substitute for potato. The growth of yeast (*Saccharomyces cerevisiae*) in broth media and molds (*Aspergillus flavus* TISTR 3366, *Bipolaris oryzae* DOAC 1760, *Fusarium semitectum* DOAC 1986 and *Penicillium* sp.) on agar media was examined. Four crops (cassava, potato, sweet potato and taro) were utilised as nutrient source in fungal media to result in four types of dextrose media while commercial potato dextrose media was used as the control. *S. cerevisiae* recorded the highest level of growth with 2.76×10^7 cells/mL when cultured in Sweet Potato Dextrose Broth at 25% sweet potato, 2% dextrose, initial pH 4.6 and agitated at 250 rpm at 27°C. Additionally, for the mold growth, Sweet Potato Dextrose Agar demonstrated significantly higher mycelial growth than commercial Potato Dextrose Agar while Taro Dextrose Agar showed similar positive result, except for *F. semitectum* DOAC 1986. This study showed that sweet potato and taro have a strong potential for use as alternative nutrient substitutes in fungal media production for yeast and mold growth.

Keywords: Culture media, fungi, growth, Potato Dextrose Agar, Potato Dextrose Broth, sweet potato, taro

INTRODUCTION

Fungi require nutrients (such as a carbon, nitrogen, vitamins, mineral elements, as well as the availability of enzymes) and certain environmental conditions (such as suitable pH value, suitable temperature, oxygen) in order to grow and reproduce. Potato has been used for fungal growth from early 20th century (Edgerton, 1908; Duggar, Severy, &

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Schmitz, 1917) and in fungal media since then (Beever & Bollard, 1970; Booth, 1971). Potato Dextrose media (PDM), made from dextrose and potato infusion, have been recognised as principal media for fungal cultivation. Fungi can break down starch in potato into soluble sugars, which can serve as a source of both carbon and energy. Furthermore, potato is a complex medium that provides nitrogen, enzymes, vitamins and mineral elements for fungal growth (Laurie, Faber, Adebola, & Belete, 2015). The high carbon: nutrient ratio of PDM hence allows efficient growth of fungi.

In most developing countries (such as Thailand), potato is generally more expensive than other cash crops. The average price per kg of potato, (obtained from the biggest wholesale farmers' market in Thailand, www. taladsimummuang.com), is higher than other crops; approximately US$ 0.99, 0.71, 0.39 and 0.34 per kg for potato, taro, cassava and sweet potato respectively (original prices are in Baht and converted into US$ with the exchange rate of 1US$ = 35.16 Baht). The cultivation of fungi using commercial PDM is costly. Therefore, researchers have attempted to develop alternative media from cassava (Weststeijn & Okafor, 1971; Rachael & Adebolu, 2014), cocoyam (Amadi & Moneke, 2012; Rachael & Adebolu, 2014), corn (Adesemoye & Adedire, 2005; Hoa & Wang, 2015), millet (Adesemoye & Adedire, 2005; Hoa & Wang, 2015), sorghum (Adesemoye & Adedire, 2005), sweet potato (Amadi & Moneke, 2012; Rachael & Adebolu, 2014; Hoa & Wang, 2015) and yam (Weststeijn & Okafor, 1971; Amadi & Moneke, 2012; Rachael & Adebolu, 2014; Hoa & Wang, 2015).

In Thailand, cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*) and taro (*Colocasia esculenta*) are widely cultivated. These cash crops not only have high carbohydrate content but are also as nutritious (they are rich in proteins, vitamins and mineral elements) as potato (Huang, Chen, & Wang, 2007; Laurie et al., 2015). Thailand is the world's biggest exporter of cassava (83% of global market share in 2009) and an important source of revenue for the country (Poramacom et al., 2013). Sweet potato is an important source of food in developing countries and is one of the seven major world staple crops. Amadi & Moneke (2012) showed that sweet potato possessed good mycelia growth when compared with yam, cocoyam and potato. Taro is a staple food in tropical and subtropical regions of the world; however, studies have yet to prove the utility of taro as fungal media.

In this study, the growth of yeast (*Saccharomyces cerevisiae*) in broth media and molds (*Aspergillus flavus* TISTR 3366, *Bipolaris oryzae* DOAC 1760, *Fusarium semitectum* DOAC 1986 and *Penicillium* sp.) on agar media was examined for possible fungal media production by using these as substitutes for potato in Potato Dextrose Media.

MATERIALS AND METHODS

Sample Collection

Cassava (*Manihot esculenta*), Potato (*Solanum tuberosum*), Sweet Potato (*Ipomoea batatas*) and Taro (*Colocasia esculenta*) were obtained from shops in Sakon Nakhon Province, Thailand.

Media formulation

Four different media were formulated, namely Cassava Dextrose Media [Cassava Dextrose Broth (CDB), Cassava Dextrose Agar (CDA)], Potato Dextrose Media [Potato Dextrose Broth (PDB), Potato Dextrose Agar (PDA)], Sweet Potato Dextrose Media [Sweet Potato Dextrose Broth (SDB), Sweet Potato Dextrose Agar (SDA)] and Taro Dextrose Media [Taro Dextrose Broth (TDB), Taro Dextrose Agar (TDA)]. The growth of yeast and molds was examined in broth media and agar media respectively. The standard method for preparing potato infusion (PDB) involved boiling 200 g of diced potato (washed and peeled) in distilled water for 30 minutes and subsequently filtered through a muslin cloth. Then, 20 g of dextrose (UNIVAR) was added to the filtrate. The volume of the mixture was measured at 1,000 mL with distilled water. The broth media were acidified with sterile 10% tartaric acid (UNIVAR) to obtain a pH value of 4.6 and were sterilised in the autoclave for 15 minutes at 121°C. This procedure was repeated in formulating CDB, SDB and TDB by substituting potato with cassava, sweet potato and taro respectively. The control medium comprised commercial Potato Dextrose Broth (Difco™) (commercial PDB).

In the agar media, the same procedure was applied but the amount of diced crop was increased to 250g, and 20g of agar (BIOMARK LABORATORIES) was added per litre. Each agar media was acidified with 10 mL of sterile 10% tartaric acid to each litre of the medium. Commercial Potato Dextrose Agar (Difco™) (commercial PDA) was used as the control medium.

Test organisms

The test yeast was *Saccharomyces cerevisiae* (Saf-instant brand, France), and the test molds were *Aspergillus flavus* TISTR 3366, *Bipolaris oryzae* DOAC 1760, *Fusarium semitectum* DOAC 1986 and *Penicillium* sp. (obtained from the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Thailand). The test organisms were maintained on PDA slants at 4°C and subcultured to fresh PDA plates regularly every month. The stocks of test organisms were subcultured and incubated at 25°C for 48 hours and 7 days for yeast and molds respectively.

The growth of yeast in broth media assay

The suitability of the formulated media (CDB, PDB, SDB and TDB) was assessed by culturing each of them with *Saccharomyces cerevisiae*. One hundred and fifty millilitres of each broth media was placed in sterilised

250 mL Erlenmeyer flasks at an initial pH value of 4.6 and inoculated with 3.0 mL of inoculum (absorbance 0.34 at wavelength 600 nm). Inoculated flasks were shaken in an orbital shaker (Thermo Scientific Forma Incubated and Refrigerated Benchtop Orbital Shakers: Model 4586) at 200 rpm for 48 hours at 25°C. The yeast cells were counted using a counting chamber (BOECO). Various concentrations of crops (50, 100, 150, 200, 250 and 300 g per litre), various concentrations of dextrose (0, 10, 15, 20, 25, 30 and 40 g per litre), various initial pH values (3.5, 4.0, 4.5, 4.6, 5.0, 5.5 and 6.0), various agitation speeds (100, 150, 200 and 250 rpm) and various temperatures (20, 25, 27, 28, 30 and 35 ºC) were investigated in a step-by-step fashion to determine optimal conditions of the test yeast to maximise the benefits of selcted crop broth media for yeast growth. Each experiment, with three replicates, was carried out.

The growth of molds on agar media assay

The selected formulated media (CDA, PDA, SDA and TDA) were evaluated by culturing them with the test molds. The test molds were subcultured to fresh PDA plates using a cork borer and incubated at 25°C for 7 days. A sterile cork borer (dipped into alcohol and flamed), with an outside diameter of approximately 6.25 mm was used to bore holes on the edge of pure starter cultures of the molds. The mycelia agar plugs were then removed using a sterilised wire needle and transferred top down onto the center of the formulated media. Each of the four molds from the pure cultures was inoculated on the plates of formulated media CDA, PDA, SDA, TDA and commercial PDA in the same manner. The diameter of growth was measured using a vernier caliper on day 7. The whole inoculation process was repeated three times for each formulated media with each of the test molds.

RESULTS AND DISCUSSION

The growth of yeast in broth media assay

Analysis of variance (ANOVA) was used to analyse results and mean differences were considered significant at $p<0.01$ by Bonferroni multiple comparison. The growth of *Saccharomyces cerevisiae* in formulating broth media is presented in Figure 1. SDB showed significantly high level of growth of *S. cerevisiae* with 6.65×10^6 cells/mL and is followed by commercial PDB, PDB, TDB

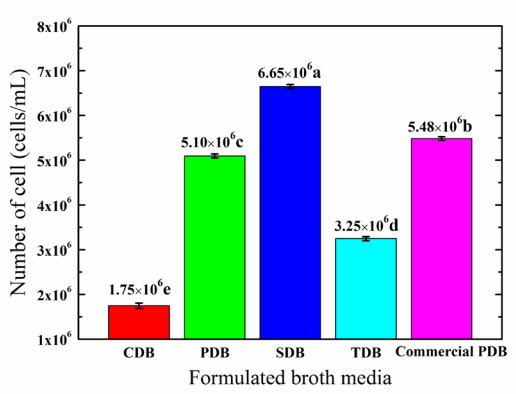

Figure 1. The growth of *Saccharomyces cerevisiae* in formulating broth media and commercial PDB over 48 h

Note. A pair of averages with different letters is considered significantly different at $p<0.01$

and CDB with 5.48×10^6, 5.10×10^6, 3.25×10^6 and 1.75×10^6 cells/mL, respectively. Laurie et al. (2015) reported that sweet potato has a higher carbohydrate and energy content than potato, and its mineral content (calcium, iron, magnesium, phosphorus, potassium and zinc) and vitamins (thiamin, niacin, riboflavin and vitamin B6) are similar with potato. The SDB could promote the highest growth of *S. cerevisiae* because carbohydrate, energy, minerals (such as iron, magnesium, phosphorus) and vitamins (especially thiamin) are key factors for fungal growth. The growth of *S. cerevisiae* in TDB was rather low. Although taro had high carbohydrate, mineral and vitamin content (Huang et al., 2007), a high amylopectin content of 72 – 83 % (Elisabeth, 2015) resulted in a sticky characteristic in the TDB which affects the oxygen absorption of yeast. The lowest growth of *S. Saccharomyces cerevisiae* was observed in CDB due to the sticky characteristic of the broth, like that of the TDB (Kwoseh et al., 2012). Trace amounts of vitamins (Laurie et al., 2015) and a high content of hydrogen cyanide (HCN) were also reported in cassava (Charles et al., 2005). Boiling of cassava will create cyanide residue in the water (Cooke & Maduagwu, 1978) as it releases HCN from its tissue (Burns et al., 2012). Although autoclaving reduced the cyanide content, it was not completely eliminated (Chove & Mamiro, 2010). It is highly toxic for all aerobic organisms including fungi because it prevents oxygen uptake (Latif & Müller, 2015).

The SDB was selected for optimising culture conditions comprising concentrations of sweet potato, concentrations of dextrose, initial pH value, agitation speeds and temperature. The growth of *S. cerevisiae* in SDB in various concentrations of sweet potato, and in various concentrations of dextrose is shown in Figures 2(a) and 2(b), respectively. The ideal concentration of sweet potato was 250 g per litre (Figure 2(a)). This concentration produced almost three times the number of cells than the standard concentration of potato infusion in Potato Dextrose Media of 200 g per litre. A concentration of dextrose at 20 g per litre demonstrated a significantly better level of growth than glucose restricted (<20 g per litre) and high glucose (>20 g per litre) cultures (Figure 2(b)). The concentration of nutrients in the culture media affected yeast growth. Glucose restriction and high glucose content could induce oxidative stress in the yeast (Francesca et al., 2010) and is capable of damaging important cellular constituents such as DNA, lipids and proteins.

The optimal pH range for yeast growth can vary from 4 to 6, depending on temperature, oxygen concentration, and strain of yeast (Narendranath & Power, 2005). The test yeast in SDB at 25% sweet potato, 2% dextrose, 200 rpm and 25°C presented the highest level of growth with an optimum pH value of 4.6 (Figure 2(c)). Noé Arroyo-López et al. (2009) discovered a similar maximum specific growth rate of *S. cerevisiae* T73 at a pH value of 4.76.

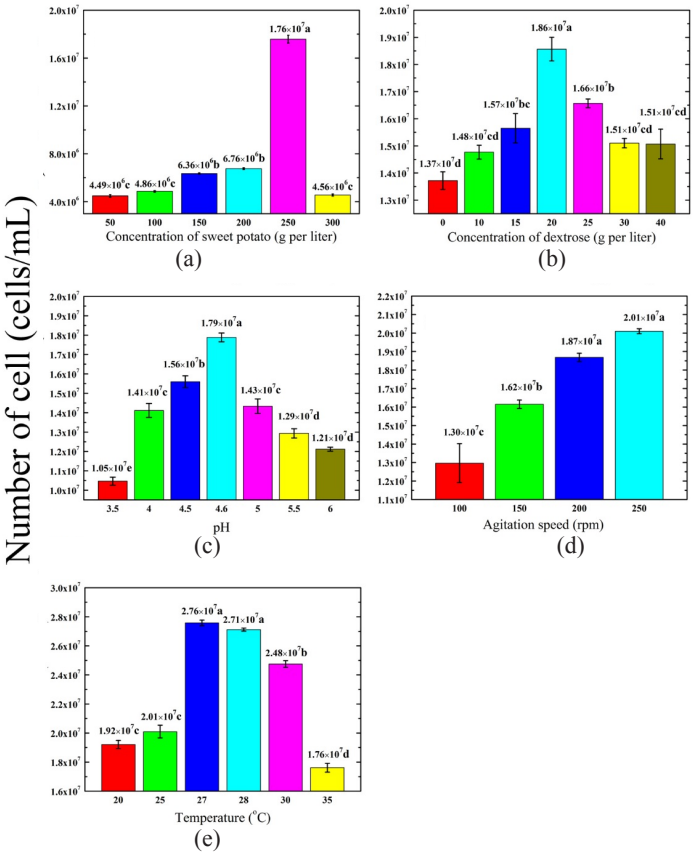

Figure 2. The growth of *Saccharomyces cerevisiae* in SDB at various culture conditions [(a) concentrations of sweet potato, (b) concentrations of dextrose, (c) initial pH values, (d) agitation speeds; and (e) temperatures] over 48 h

Note. A pair of averages with different letters is considered significantly different at $p<0.01$

In aerobic organisms, the two major functions of respiration are oxidation of reduced cofactors and the generation of metabolic energy in the form of ATP. Oxygen is used as the terminal electron acceptor for mitochondrial respiration and for assimilatory oxygenation reactions (Weusthuis et al., 1994). It is an important factor in yeast metabolism, which could clearly be seen in the increase in agitation speed from 100 to 250 rpm that led to an increase in the yeast population (Figure 2(d)). However, agitation speeds of 200 and 250 rpm did not affect the number of yeast cells.

The growth of *S. cerevisiae* in SDB at 25% sweet potato, 2% dextrose, an initial pH value of 4.6 and at 250 rpm at various temperatures is shown in Figure 2(e). The best growth of the test yeast was achieved at 27°C as reported by Pérez-Ramírez et al. (2012) who found that the maximum level of biomass production of *S. cerevisiae* in coconut water medium was obtained at 27°C, while temperatures higher or lower than 27°C caused a decrease in biomass

values. Nevertheless, in this study, the test yeast illustrated the best level of growth at 28°C with no significant difference at 27°C. The lowest level of growth was recorded at 35°C. High temperature stress causes many changes in the yeast cells that can ultimately affect protein structures and functions, accumulate denatured and aggregated biomacromolecules, and give rise to growth inhibition or cell death. It can also disorder the integrity of cell membranes, increase membrane permeability, and affect the plasma membrane fluidity (Zhang et al., 2015).

The growth of molds on agar media assay

The growth of the four test molds on four samples of formulated agricultural crop agar media and commercial PDA over seven days are presented in Figure 3. *Aspergillus flavus* TISTR 3366 grew significantly fastest on SDA and TDA with a growth diameter of 80.35 and 80.26 mm respectively. Rachael and Adebolu (2014) reported a similar best mycelial growth of *A. flavus* in SDA whereby the latter also promoted significantly higher mycelial growth of *Fusarium semitectum* DOAC 1986 and *Penicillium* sp. than on the other formulated agar media, including commercial PDA. *Bipolaris oryzae* DOAC 1760 showed the highest level of growth on TDA. A comparison of mold growth on the formulating agar media with commercial PDA revealed that all of the test molds grew significantly higher on SDA than on commercial PDA, while most of the test molds grew significantly higher on TDA than they did on commercial PDA, except for *F. semitectum* DOAC 1986. However, both SDA and TDA stimulated higher mycelial growth than PDA in all of the test molds. Similar results of fungal growth enhancement were found in oyster mushroom (*Pleurotus cystidiosus*) with Sweet Potato Dextrose Agar (Hoa & Wang, 2015). Sweet potato was excellent in encouraging either the growth of the test yeast or the growth of the test molds. The sticky characteristic of the taro on fungal growth has an effect on the broth media but it does not affect the agar media. The lowest growth of mycelium in all test molds was found on CDA. This was to be expected because of the low vitamin content in cassava. The HCN was also toxic for both the test yeast and molds. Commercial PDA showed higher levels of all test molds growths than PDA.

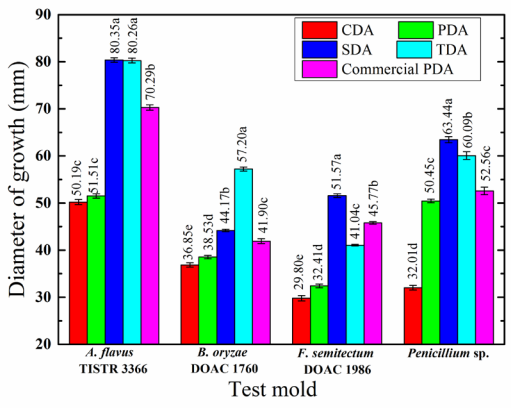

Figure 3. The mycelial growth of test molds on formulating agar media and commercial PDA over 7 days

Note. A pair of averages in each test molds with different letters is considered significantly different at $p<0.01$

CONCLUSION

Our findings showed that sweet potato can be a good alternative source for potato in Potato Dextrose Media, whereas taro can only be used as a substitute for the potato on agar media for yeast and mold cultivation, especially with regard to biomass production. Crops with high content of amylopectin, that cause sticky characteristic, are not appropriate for use in liquid media. Tropical and subtropical countries which has good laboratory facilities can use sweet potato and taro as substitutes for potato in fungal media production.

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