

Research Article

Screening of ethanol producing yeasts and bacteria in dried longan extract for the synthesis of *R*-phenylacetylcarbinol

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Abstract

An alternative strategy for solving the problem of more than 67,000 tonnes of deadstock dried longan in the Northern region of Thailand since 2004 was to use it as a carbon source for bioconversion of sugars to ethanol and to employ the generated microbial biomass for biotransformation of pyruvate and benzaldehyde to *R*-phenylacetylcarbinol (PAC) – a precursor for pharmaceutical compounds such as ephedrine and pseudoephedrine. Three types of carbon sources, namely, expired portion of dried longan aged 5 years old, dried longan aged 1 month old and dried longan aged 1 month old mixed with molasses in 1 : 1 ratio were used in the current study for the screening of whole cells biocatalyst from 15 microbial strains in 100 ml scale for subsequent whole cells production in the larger scale (1,000 ml) and PAC biotransformation based on a biphasic system. The expired portion of dried longan extract aged 5 years old was not suitable for the production of ethanol and dried biomass for biotransformation. The screening results of an appropriate carbon source and a microbial strain for large scale production of whole cells biocatalyst were dried longan aged 1 month old mixed with molasses in 1 : 1 ratio and *Saccharomyces cerevisiae* TISTR 5606. The study on ethanol production kinetics and whole cells production of *S. cerevisiae* TISTR No. 5606 in a steady state condition of 1,000 ml for 72 h showed that 47.3 ± 1.5 g/l ethanol was generated with corresponding ethanol yield of 0.32 ± 0.03 g ethanol produced/g

sugars consumed. The effect of various primary alcohol species to PAC production in a two-phase system with whole cells biocatalyst harvested at 72 h from the previous experiment indicated that the application of octanol yielded the highest PAC concentration of 10.4 ± 0.5 mM. In addition, dipropylene glycol exerted a positive effect on PAC production when mixed with short chain (methanol, ethanol and propanol) as well as long chain alcohols such as heptanol.

Keywords: *Dimocarpus longan*, biotransformation, biphasic system, *Saccharomyces cerevisiae*, pyruvate, benzaldehyde, PAC, Thailand.

Introduction

Longan has been one of the important economic crops of Thailand, second only to pineapple (export value of \$US361 million), durian (\$US87 million) and baby corn (\$US58 million) [1]. Each year, Thailand exports fresh longan to various countries with an annual value of about \$US57.8 million [1]. Thirty percent of fresh longan production is consumed domestically, while more than 40% of the overall produce are processed and the remainder exported [2]. In 2006, the overall export value of longan in northern Thailand was up to \$US112 million, which was higher than lychee and orange by a factor of approximately 6 and 13 times, respectively [1]. Choo [3], stated that fresh longan contained all crucial ingredients which could support microbial growth such as 25.2% (w/w) carbohydrate, 1.0% (w/w) protein, minerals and other vitamins. After the drying process, the moisture content of fresh longan is decreased from 72.4% (w/w) [3] to 12–18% (w/w) [4].

Molasses from sugar production is another type of raw material which is commonly used for ethanol production and was recently subjected to an intense investigation in various systems such as immobilized cells in fluidized bed [5] and cell recycle [6].

The problem of deadstock dried longan began in 2004 and is still considered as an unsolved issue until the present day. Dried longan is a popular ingredient in Chinese cuisine and it used to be regularly exported from northern Thailand to southern China. In attempts to assist the industry following a free trade agreement with China, the government at the time offered guaranteed prices or subsidies and was stockpiling dried longan. This mortgage scheme was tainted by politics and a glut of dried longan resulted. Initially attempts were made to produce fertilizer however, much of the stockpile proved to be too degraded to make this a viable option. Thus the possibility of producing ethanol was considered. According to FreshPlaza [7], the overall amount of 67,000 tonnes deadstock dried longan was being maintained in 115 storage facilities across six northern provinces of Thailand which included Chiang Mai, Chiang Rai, Lamphun, Lampang, Payao and Nan. One alternative solution to this matter was the bioconversion of leftover sugars in dried longan to ethanol by an appropriate microbial strain [8, 9]. The ethanol containing supernatant could be distilled and filtered to obtain high purity grade ethanol while the remnant microbial biomass with pyruvate decarboxylase (PDC) enzyme was subsequently utilized for the biotransformation of pyruvate and benzaldehyde to *R*-phenylacetylcarbinol (PAC) in a well-known Knoll procedure [10, 11] as illustrated in Figure 1. PAC is a chiral precursor for the production of pharmaceutical substances, namely, ephedrine and pseudoephedrine. The estimated costs of both medicines in the hydrochloride form by FDA Thailand [12] were 4,450 and 2,000 Baht/kg, respectively. Numerous PAC production systems had been investigated, for example, live yeast cells in batch [13, 14], fed batch or continuous systems [15, 16], partially purified PDC [17] and frozen whole cells of yeast [18].

There were three objectives of this research. Firstly, an appropriate microbial strain for production of ethanol and biomass was screened. This was followed by the selection of a suitable cultivation medium for the production of a whole cell catalyst. Lastly, biphasic biotransformation of PAC in different types of organic phase was carried out for the selected whole cell biocatalyst. The current system employed dried longan instead of glucose and replaced buffer species in the biotransformation process from the previously described 3 - (N - morpholino) propanesulfonic acid (MOPS) buffer [17, 18] (88,400 Baht/kg) [19] to the much cheaper phosphate buffer (840 Baht/kg).

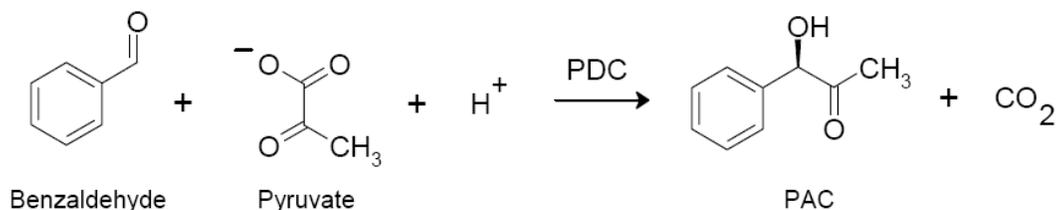


Figure 1: Biotransformation of PAC from pyruvate and benzaldehyde [20].

Materials and Methods

Microorganisms

The following 15 microbial strains were ordered from Thailand Institute of Scientific and Technological Research (TISTR); six strains of *Candida utilis* (TISTR 5001, 5032, 5043, 5046, 5198 and 5352), two strains of *Escherichia coli* (TISTR 361 and 1261), one strain of *Klebsiella* sp. (TISTR 1383), three strains of *Saccharomyces cerevisiae* (TISTR 5020, 5339 and 5606) and three strains of *Zymomonas mobilis* (TISTR 405, 548 and 550). Each microbial strain was propagated and stored in a frozen glycerol stock at $-20^{\circ}C$.

Chemicals, media and preparation

All chemicals used in the experiment were analytical grade. The media was sterilized at $121^{\circ}C$, 15 psi for 15 min with a portable pressure sterilizer (All American, Model No. 1925x, Wisconsin, United States). Five molar KOH was used in the pH adjustment.

Inoculum Media

Nutrient broth for *E. coli* and *Klebsiella* sp. consisted of (in one litre): 3.0 g beef extract and 5.0 g peptone. Yeast medium for *C. utilis* and *S. cerevisiae* composed of (in one litre): 10.0 g glucose, 3.0 g yeast, 5.0 g malt extract and 5.0 g peptone. *Zymomonas* medium for *Z. mobilis* consisted of (in one litre): 20.0 g glucose, 10.0 g yeast extract and 10.0 g peptone [21].

Concentrated Dried Longan Extract

Twenty litres of distilled water was heated until boiled and followed by the addition of 6 kg dried longan aged five years old (Kandee Agriculture, Lamphun) or one month old (Amphur Sanpatong, Chiang Mai) at room temperature. The dried longan aged five years old was obtained from the spoiled portion which constituted approximately 10% of the overall stock. The boiling was continued for the next 30 min. The container was cooled down at room temperature for 2.5 h before the filtration step was carried out through sieves whose openings were $1 \times 1 \text{ mm}^2$ and $0.5 \times 0.5 \text{ mm}^2$, respectively. The pH of the longan extract was adjusted to 6.5. In the case of one month old dried longan, additional nitrogen sources were introduced

which included (in one litre): 4.5 g yeast extract, 7.5 g malt extract and 7.5 g peptone. The concentrated extract from one month old dried longan extract was pre-diluted to 21-22°Brix prior to the addition of nitrogen source [23, 31]. The omission of nitrogen source addition from the five year old dried longan extract medium was to assess the ability of this medium alone to sustain the growth of the screening microbes.

Concentrated Molasses

Two hundred grams of molasses was dissolved in distilled water until the final volume of 1 litre was reached. The pH of this solution was adjusted to 6.5.

Cultivation Medium Mixture of Dried Longan Extract and Molasses

The concentrated longan extract from dried longan aged one month old was mixed with the concentrated molasses in 1 : 1 ratio for the cultivation in both 100 ml and 1,000 ml scale with the addition of extra nitrogen source as follows (in one litre): 4.5 g yeast extract, 7.5 g malt extract and 7.5 g peptone.

Cultivation of microbes

Preparation of Microbial Inocula

The frozen stock of 0.8 ml for each microbial strain was thawed and added to 10 ml of corresponding inoculum medium for the later cultivation in 100 ml scale for either microbial screening or subsequent kinetics assessment and whole cell production in 1,000 ml scale. The cultivation of each inoculum was carried out for 48 h at 25.6°C which was the average annual temperature of Chiang Mai Province between 1988 – 2005 [22].

Cultivation of Microbial Culture in 100 ml Scale

The prepared microbial inocula in the previous step was added to the following 100 ml cultivation media in 550 ml glass bottle for either the screening purpose; (1) dried longan extract aged five years old, (2) dried longan extract aged one month old with additional nitrogen sources and (3) the mixture between dried longan extract aged one month old and molasses in 1:1 ratio with additional nitrogen sources, or the application of medium (3) as seed culture for subsequent kinetics assessment and whole cell production in 1,000 ml scale. The microbial cultivation in medium (1) was done in an aerated condition through a fish tank pump for 24 h before switching off the pump and cultivation in static conditions for another 24 h. The total cultivation period in all screening experiments was 48 h at 25.6°C at static condition (unless stated otherwise) in duplicate.

Cultivation of Microbial Culture in 1,000 ml Scale

The seed culture of the selected microbial strain obtained from 100 ml scale in medium (3) was transferred to 2,000 ml bottle with 1,000 ml cultivation medium of the same type. The collection of samples was carried out at a regular interval of 7 or 9 h from time 0 until 72 h (0, 7, 14, 21, 28, 35, 42, 49, 56, 65 and 72 h). The culture was frozen rapidly in liquid nitrogen and maintained at -20°C until the collection of whole cell biocatalyst at 72 h was carried out with centrifugation at $2,822 \times g$ for 15 min (Sorvall centrifuge machine, Model Super T21) so that biotransformation experiment could be performed at the later stage.

Biphasic biotransformation

The whole cell biocatalyst obtained from the cultivation in 1,000 ml scale was adjusted to 3.06 g/l (dried biomass equivalent) in 900 mM phosphate buffer (pH 6.5) and used to investigate the influence of seventeen organic solvents in biphasic PAC biotransformation.

These organic compounds included; the pure solvent of (1) methanol (C1), (2) ethanol (C2), (3) propanol (C3), (4) butanol (C4), (5) pentanol (C5), (6) hexanol (C6), (7) heptanol (C7), (8) octanol (C8), (9) nonanol (C9), (10) decanol (C10) and (11) dipropylene glycol (DPG), as well as six solvent mixtures of (12) DPG + C1, (13) DPG + C2, (14) DPG + C3, (15) DPG + C7, (16) DPG + C8 and (17) DPG + C9. The organic phase contained 500 mM of benzaldehyde in the specified solvent of 5 ml volume. The equivalent volume of aqueous phase contained 600 mM pyruvate, 1 mM thiamine pyrophosphate (TPP), 1 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, phosphate buffer and 3.06 g/l whole cell biocatalyst. The biotransformation was done at 6°C for 24 h in an orbital shaking incubator (n-Biotek, Model NB-205) at 200 rpm. The experiment was carried out in duplicate.

Analytical methods

For the fermentation process, the cell pellet was analyzed for dried biomass [23] while the optical density of the biomass was measured at 600 nm (OD600). The supernatant was analyzed for pH, total soluble solid (TSS) in degree Brix with a hand refractometer (Atago, N-1 α). The determination of sugars (sucrose, glucose and fructose), organic acids (citric and acetic acid), and alcohol (ethanol) was performed with high performance liquid chromatography (HPLC) using Aminex® HPX-87H column [23, 24, 25]. The analyses were carried out with four replicates. For the biotransformation process, the determination of PAC was carried out with Alltima™ C8 5 μm column using the method of Rosche *et al.* [26]. Each analysis was done in triplicate. The statistical hypothesis testing was computed based on t-test method of Skoog *et al.* [27] with VBA6.3 program in Microsoft® EXCEL2003.

Results and Discussion

Screening of carbon source and whole cell biocatalyst

The dead portion of dried longan extract aged 5 years old was not suitable for the production of ethanol as evident from the relatively low average initial concentration of glucose (0.13 ± 0.03 g/l, Fig. 2(a)), fructose (1.26 ± 0.07 g/l, Fig. 2(b)) and sucrose (0.63 ± 0.10 g/l, Fig. 2(c)) as well as the minute quantity of produced ethanol (less than 0.1 g/l, Fig. 2(d)). However, the expired portion of dried longan in the current study constituted only 10% of the overall dried longan aged 5 years old [28]. Further study of the remnant dried longan aged 5 years old which contained a higher concentration of sugars is necessary in the future. A number of microbes were able to thrive in the sugar depleted conditions as follows (Fig. 2(e)); *Klebsiella* sp. TISTR 1383 (4.81 ± 0.67 g/l), *C. utilis* TISTR 5198 (3.47 ± 0.26 g/l), *E. coli* TISTR 361 (3.42 ± 0.77 g/l), *S. cerevisiae* TISTR 5606 (2.41 ± 0.19 g/l) and *C. utilis* TISTR 5043 (2.29 ± 1.45 g/l).

The comparison of glucose consumption of 15 microbial strains in dried longan extract aged 1 month old (Fig. 3(a)) and the mixture of dried longan extract aged 1 month old and molasses in 1:1 ratio (Fig. 4(a)) revealed five microbial strains, namely, *C. utilis* TISTR 5198, *S. cerevisiae* TISTR 5020, *S. cerevisiae* TISTR 5606, *Z. mobilis* TISTR 405 and *Z. mobilis* TISTR 550 which were able to consume all glucose until the final concentration after 48 h was less than 0.2 g/l. On the contrary, there were six microbial strains with remnant glucose concentration levels of more than 24 g/l, which included *C. utilis* TISTR 5001, TISTR 5032, TISTR 5043, TISTR 5046, TISTR 5352 and *Z. mobilis* TISTR 548 (Fig. 4(a)). These two groups of microbial strains could not use fructose in an efficient manner as glucose which was evident from the higher concentration level of fructose after 48 h cultivation period with the exception of *Z. mobilis* TISTR 548 whose fructose concentration

level was lower than that of glucose in both types of cultivation media (Fig. 3(b) and 4(b)). There might be evidence of invertase (sucrase) enzyme activity [29] which hydrolyzed sucrose to fructose and glucose from the apparent increase of glucose and fructose concentration levels in the media after 48 h cultivation period. From Fig. 3(c) and 4(c), there were six microbial strains which lacked the ability to consume sucrose effectively such as *C. utilis* TISTR 5198, *E. coli* TISTR 361, *E. coli* TISTR 1261, *Klebsiella* sp. TISTR 1383, *S. cerevisiae* TISTR 5339 and *Z. mobilis* TISTR 405.

The reason for selecting *S. cerevisiae* TISTR 5606 instead of *S. cerevisiae* TISTR 5020 for the production of whole cell biocatalyst in 1,000 ml scale was based not only on the ability of this yeast strain in producing a high concentration of ethanol, but also dried biomass. According to Fig. 3(d), 3(e), 4(d), 4(e), and Table 1, the highest level of the multiplication between the concentration of produced ethanol and dried biomass was 181 ± 27 (g/l)² for *S. cerevisiae* TISTR 5606 with the cultivation medium containing dried longan extract and molasses in 1:1 ratio. The cultivation medium from dried longan extract aged 6 years old was excluded due to its relatively low sugar content.

Table 1: Criteria used in the selection of appropriate carbon source for cultivation medium and yeast strain for the production of whole cell biocatalyst in 1,000 ml scale.

Yeast Strain (TISTR)	Type of Carbon Source					
	Dried Longan Extract Only			Dried Longan Extract & Molasses		
	ΔP (g/l)	ΔX (g/l)	$\Delta P * \Delta X$	ΔP (g/l)	ΔX (g/l)	$\Delta P * \Delta X$
5020	45.0 ± 6.1	2.76 ± 1.10	124 ± 52	43.3 ± 4.0	3.79 ± 0.58	164 ± 29
5606	37.8 ± 3.1	4.17 ± 2.46	158 ± 94	41.6 ± 1.2	4.35 ± 0.63	181 ± 27

In terms of dried biomass production after 48 h cultivation period, the highest dried biomass producers in dried longan extract aged one month old (Fig. 3(e)) included (produced dried biomass concentration) *S. cerevisiae* TISTR 5606 (4.17 ± 2.46 g/l), *S. cerevisiae* TISTR 5020 (2.76 ± 1.10 g/l), *C. utilis* TISTR 5198 (2.58 ± 1.85 g/l) and *S. cerevisiae* TISTR 5339 (2.53 ± 0.73 g/l). This was compared to the growth in the mixture of dried longan and molasses as follows; *S. cerevisiae* TISTR 5606 (4.35 ± 0.63 g/l), *S. cerevisiae* TISTR 5339 (4.12 ± 1.04 g/l), *C. utilis* TISTR 5198 (4.08 ± 1.01 g/l) and *S. cerevisiae* TISTR 5020 (3.79 ± 0.58 g/l).

The level of acetic and citric acid concentrations from 15 microbial strains in all three types of carbon source were less than 1.5 g/l (data not shown).

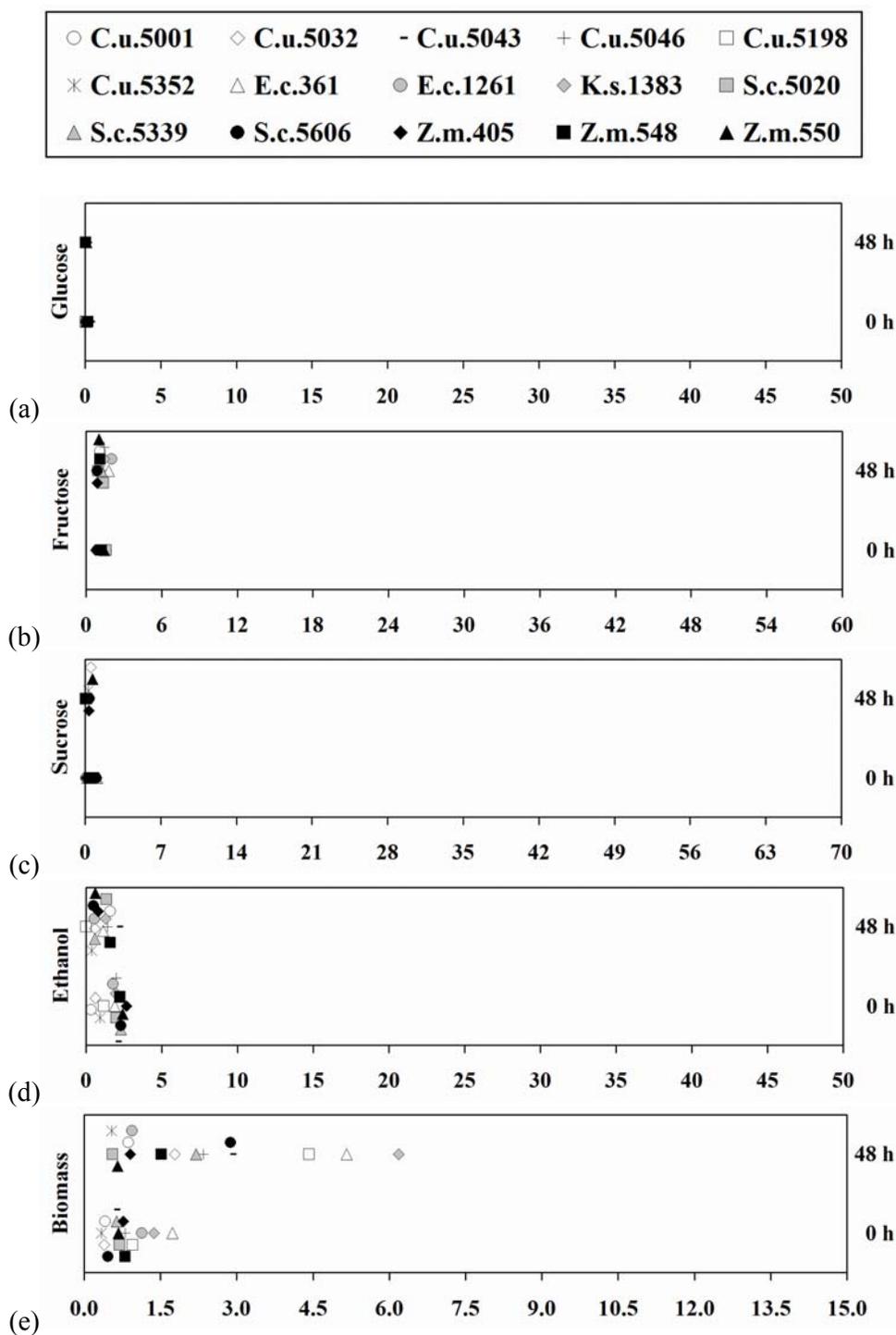


Figure 2: Cultivation of 15 microbial strains in dried longan extract aged 5 years old in the aerated condition for 24 h which was followed by static condition for another 24 h. X-axes units are in g/l. Y-axis describes the detected concentrations after inoculation for at 0 h and 48 h.

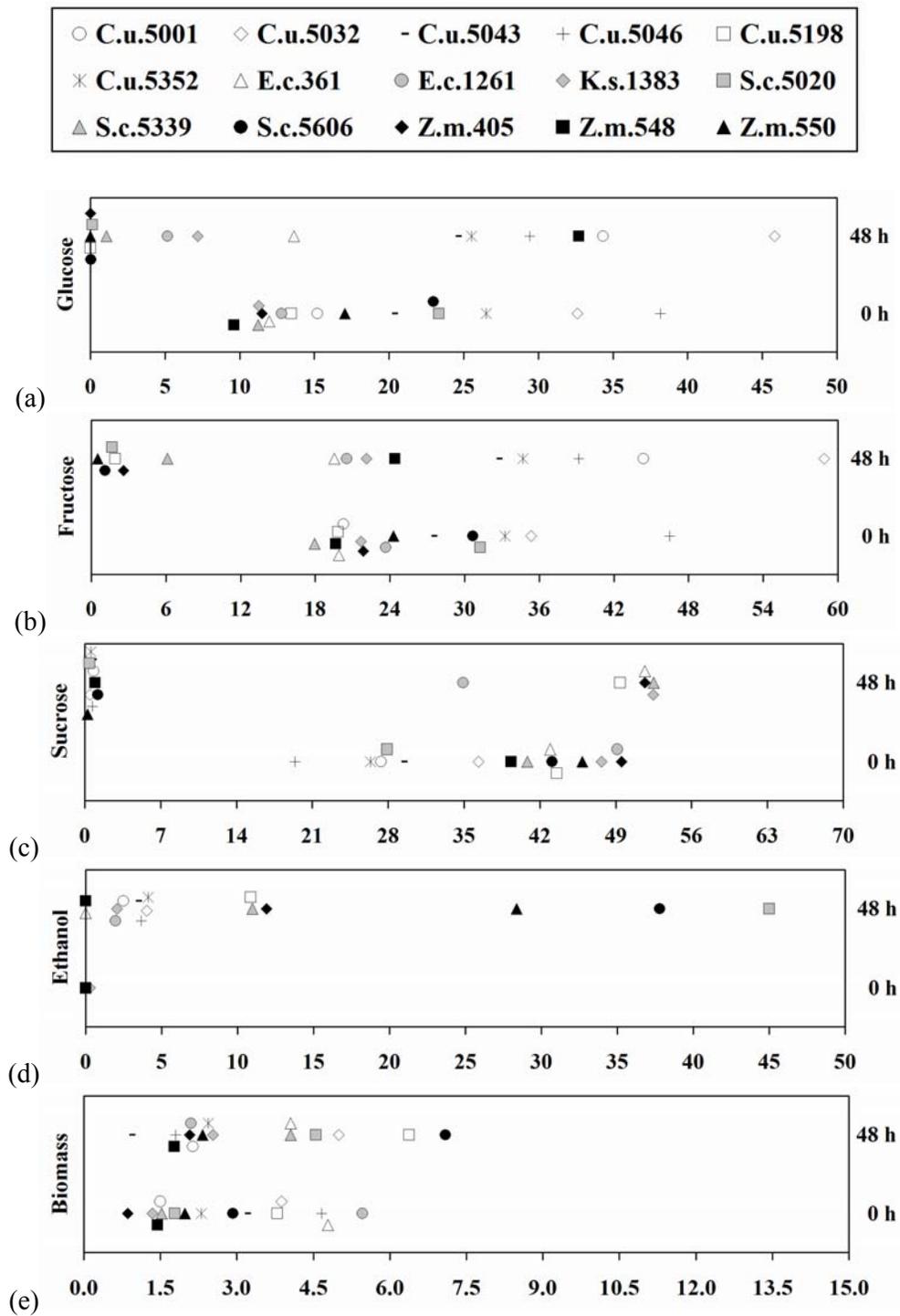


Figure 3: Cultivation of 15 microbial strains in dried longan extract aged 1 month old with addition of nitrogen source in static condition for 48 h. X-axes units are in g/l. Y-axis describes the detected concentrations after inoculation for at 0 h and 48 h.

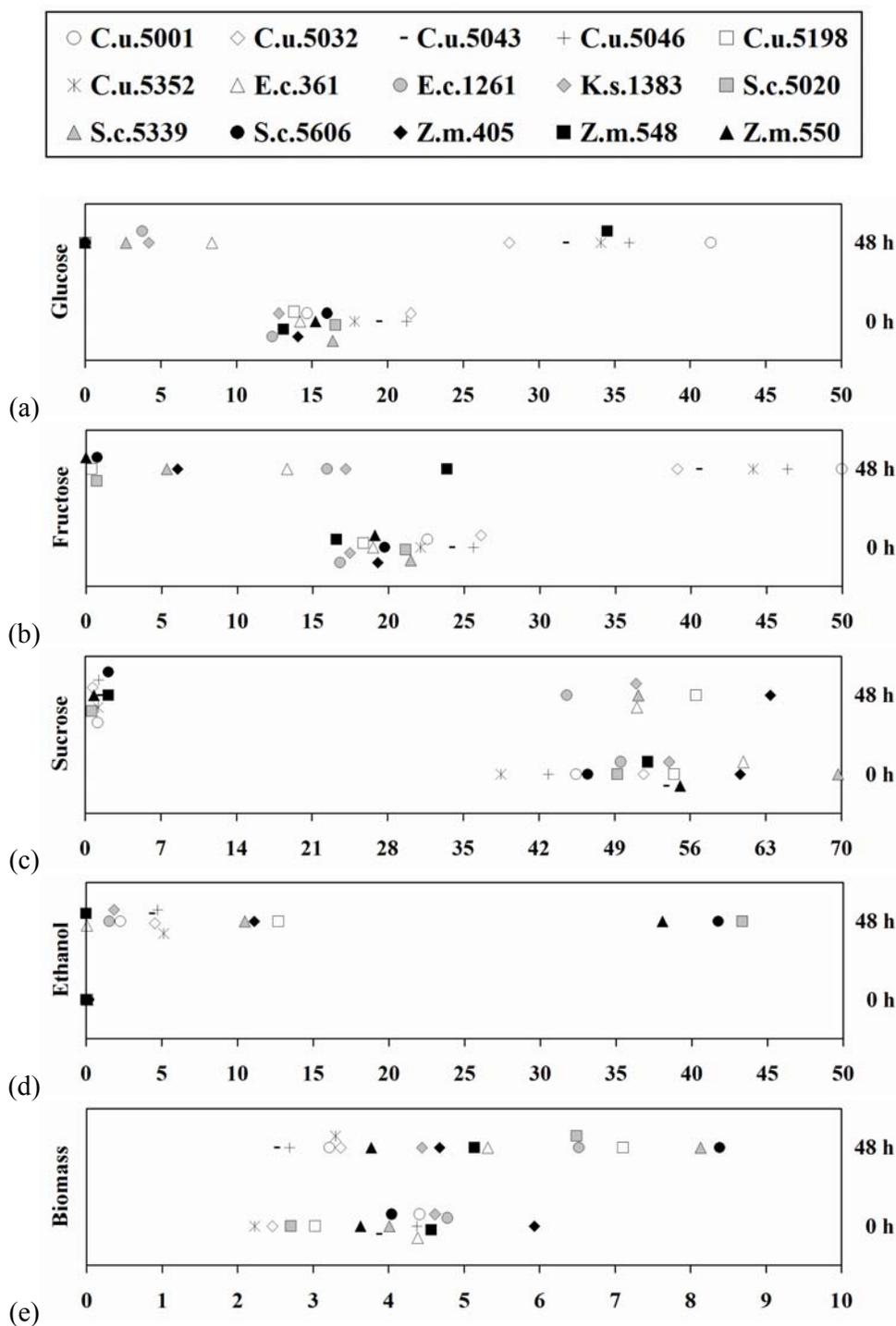


Figure 4: Cultivation of 15 microbial strains in the mixture of dried longan extract aged 1 month old and molasses in 1:1 ratio with addition of nitrogen source in static condition for 48 h. X-axes units are in g/l. Y-axis describes the detected concentrations after inoculation for at 0 h and 48 h.

Production of *S. cerevisiae* TISTR 5606 whole cell biocatalyst

The kinetic parameters obtained from the fermentation profiles in Fig. 5(a) and 5(b) were evaluated during the first 21 h and all profiles in both figures, except pH value, reached the plateau after 28 h of cultivation. The kinetics for sugar consumption and ethanol product is shown in Fig. 5(a) without the presence of lag phase. The ethanol yield over the consumed total sugar at 28 and 72 h were 0.32 ± 0.02 and 0.32 ± 0.03 g ethanol produced per g consumed sugar, respectively. The specific sucrose, fructose and glucose consumption rates were 1.31 ± 0.79 g sucrose per g dried biomass per h, 0.30 ± 0.13 g fructose per g dried biomass per h and 0.29 ± 0.17 g glucose per g dried biomass per h. The specific rate of ethanol production was 0.43 ± 0.08 g ethanol per g dried biomass per h. Fig. 5(b) illustrated the kinetics of TSS, OD600, pH and dried biomass. The rate of decrease in TSS value was 0.71 ± 0.25 degree Brix per h while the rate of increase in OD600 was 1.12 ± 0.08 units per h. The cultivation medium used in the current study had the ability to withstand pH change as evident from a relatively constant pH value of 4.98 ± 0.03 which might stem from the presence of buffering species within the dried longan extract and molasses [30]. The specific growth rate and doubling time were determined to be 0.083 ± 0.031 per h and 8.35 ± 3.08 h, respectively.

Biphasic biotransformation of PAC

The concentration of PAC in each phase for the specific organic solvent/phosphate buffer system is given in Table 2, with the overall PAC concentration which took into account the volume ratio of each phase in Figure 6. From Table 2, the examined biotransformation systems could be classified into two groups as follows. The first group belonged to the system which utilized C1, C2, DPG, DPG + C1, DPG + C2 and DPG + C3 as their organic phases. There was no clear distinction between organic and aqueous phases after each sample was collected at 24 h and passed through centrifugation procedure. The situation was different in the second group (C3 – C10, DPG + C7, DPG + C8 and DPG + C9) where the appearance of a biphasic layer was evident. The PAC concentration in the first group was at the highest level (7.85 ± 1.58 mM) and differed statistically ($p \leq 0.05$) from other members in this group when DPG was used as an organic solvent. The addition of methanol (C1) seemed to inhibit the formation of PAC while the detected concentration in the system with DPG + C2 and DPG + C3 was not significantly different ($p > 0.05$). In the system with an obvious separation of liquid layers, the PAC concentration in the organic phase was always significantly higher ($p \leq 0.05$) than the concentration in the aqueous phase (except C4 where PAC formation was not detected in either phase). This was due to the hydrophobic nature of PAC which tended to mingle well with the organic phase as illustrated from its structure in Fig. 1. The highest PAC concentration in an aqueous phase was 4.20 ± 0.16 mM for DPG + C9 which was not significantly different ($p > 0.05$) from the systems with DPG + C7 and DPG + C8. Further comparison of the produced PAC concentration in the organic phase revealed that C8 was an optimal system which was in agreement with findings from Rosche *et al.* [26]. The highest PAC concentration in this system was 19.6 ± 0.4 mM which was closely followed by the system which adopted DPG + C7, DPG + C8 and DPG + C9 as their organic phases with concentration range of PAC between 16.2 – 16.9 mM.

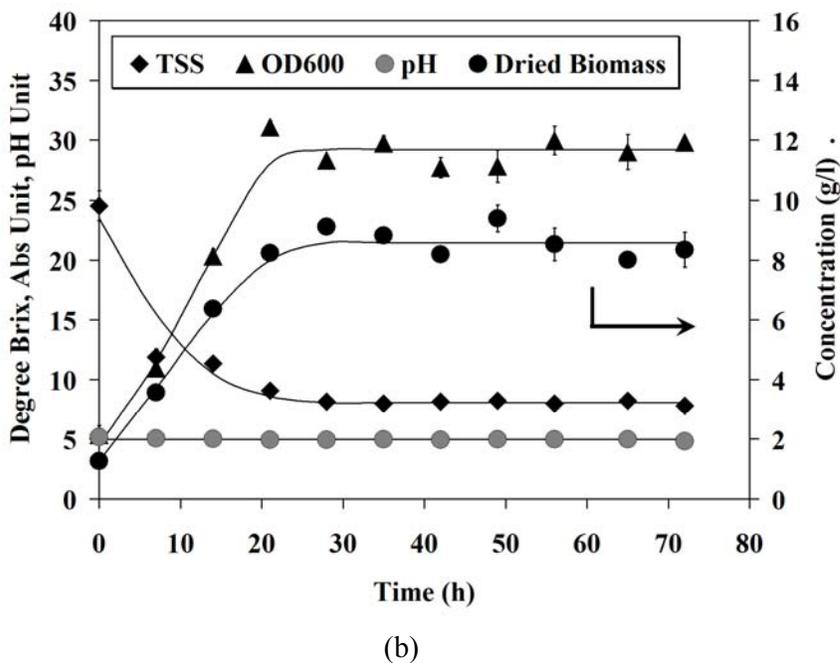
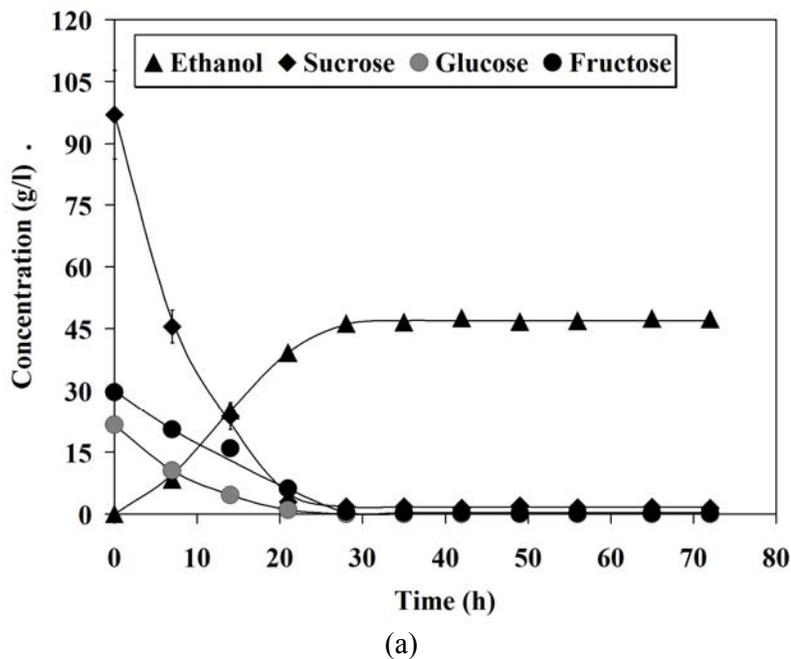


Figure 5: The kinetics of (a) substrate consumption and ethanol production as well as (b) total soluble solid (TSS), optical density at 600 nm (OD600), pH and dried biomass concentration for *S. cerevisiae* TISTR 5606 in 1,000 ml scale.

Table 2: The determined concentration of PAC in organic and aqueous phases which corresponded to each biotransformation system.

Type of organic solvent	[PAC] (mM)					
	Aqueous			Organic		
C1*						
C2*						
C3	0.08 ± 0.00	A	I	0.75 ± 0.00	A	II
C4	0.00 ± 0.00	B	I	0.00 ± 0.00	B	I
C5	0.00 ± 0.00	B	I	15.0 ± 0.3	C	II
C6	0.64 ± 0.00	C	I	10.3 ± 0.2	D	II
C7	1.37 ± 0.10	D	I	14.9 ± 0.01	C	II
C8	1.76 ± 0.03	E	I	19.6 ± 0.4	E	II
C9	1.61 ± 0.01	D	I	13.1 ± 1.0	C	II
C10	1.31 ± 0.12	D	I	9.80 ± 0.55	D	II
DPG*				7.85 ± 1.58 (b)		
DPG + C1*				1.32 ± 0.06 (c)		
DPG + C2*				2.62 ± 0.30 (d)		
DPG + C3*				2.40 ± 0.20 (d)		
DPG + C7	3.12 ± 0.77	D,E,F	I	16.4 ± 0.6	C,F	II
DPG + C8	3.80 ± 0.01	F	I	16.2 ± 0.2	F	II
DPG + C9	4.20 ± 0.16	F	I	16.9 ± 0.1	F	II

The numbers with the same alphabet (A-F) & ((a)-(d)), for comparison between each row of the same column, or Roman numeral (I-II), for comparison between each column of the same row, indicated no significant difference ($p > 0.05$). The asterisk (*) indicated the system without the distinct phase separation after centrifugation.

The average concentration of PAC in Fig. 6 illustrated clearly the beneficial role of DPG in PAC production. In the absence of DPG, the systems which utilized C1 and C2 as their organic phases did not produce any detectable quantity of PAC while the application of C3 resulted in the overall PAC concentration of only 0.60 ± 0.03 mM. The addition of DPG to these solvents in 1:1 ratio resulted in significant improvement ($p \leq 0.05$) of PAC concentration to the level of 1.32 ± 0.09 , 2.62 ± 0.32 and 2.40 ± 0.23 mM, respectively. The similar effect was less pronounced when DPG was introduced to the long chain alcohol (C7 – C9). The positive significant difference ($p \leq 0.05$) was only observed for C7, whereas the difference was not significant ($p > 0.05$) for C9 and even negative significant difference ($p \leq 0.05$) was exhibited for C8. The highest overall concentration of PAC was 10.4 ± 0.5 mM in the system with C8 as an organic phase which was not found to be significantly different from the system with DPG + C7.

The investigation of a biphasic biotransformation by Poodtatep *et al.* [31] with 500 mM benzaldehyde in an octanol phase at 4°C for 24 h in the static condition with 3.06 g/l (dried biomass equivalent) of *S. cerevisiae* 5606 as a biocatalyst revealed the PAC production level of only 1.74 ± 0.42 mM, which was less than the current study by almost 6 times. However, the PAC concentration level produced herewith was still lower than that of Leksawasdi *et al.* [17] who utilized the partially purified PDC from *C. utilis* strain UNSW 70940 or WCCN 248 as a biocatalyst in the biphasic system (2.5 M DPG in C8/20 mM MOPS buffer) with an automatic pH control system and overhead stirrer speed setting of 255 rpm. The reported level of PAC in this system was 613 mM in 47 h.

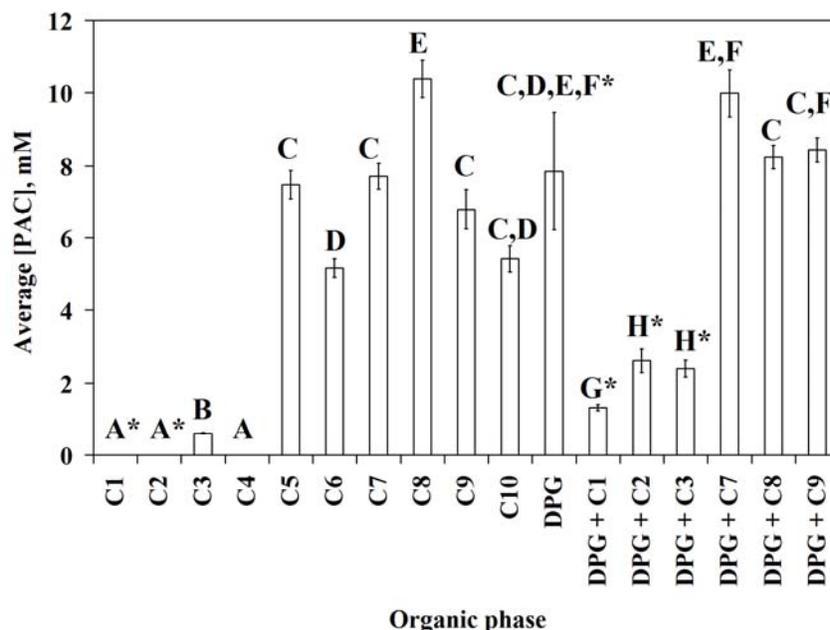


Figure 6: The average concentration of PAC in both organic and aqueous phases obtained after 24 h of biotransformation period in seventeen types of organic solvents. The same alphabetical letter above each bar indicated no significant difference ($p > 0.05$). The asterisk (*) signified the biotransformation system where the separation into biphasic layers was not observed after centrifugation.

Conclusion

The expired portion of dried longan which constituted 10% of dried longan aged 5 years old in the deadstock was not suitable for the production of ethanol and dried biomass for subsequent PAC biotransformation. Further study on the remaining dried longan which contained a higher level of sugar in the deadstock is necessary. The mixture between dried longan extract aged 1 month old and molasses in 1:1 ratio was the most appropriate carbon source for the cultivation of *S. cerevisiae* TISTR 5606 in 1,000 ml scale. Octanol was the best solvent for biphasic biotransformation with whole cell biocatalyst of 3.06 g/l (dried biomass equivalent) and resulted in the highest PAC concentration of 10.4 ± 0.5 mM.

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