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Research Article

A Potential of Mutant Yeast Strain for Improvement Arabica Coffee Fermentation Process

Yaowapa Meeampun[1], Prapakorn Tantayotai[1], Titiporn Panyachanakul[2], Siritron Samosorn[3], Kulvadee Dolsophon[3], Rossaporn Jiamjariyatam[3], Wanlapa Lorliam[1], Jantima Arnthong[4], Surisa Suwannarangsee[4] and Sukhumaporn Krajangsang[1*]

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ABSTRACT

Arabica coffee is a worldwide popular beverage. Coffee fermentation is important process to enhance the coffee flavor quality. Microorganisms involved in the process are the main factor affecting coffee quality. This study aims to apply the new starter culture of *Wickerhamomyces anomalus* UV22-3, a UV mutant strain, for coffee fermentation and to improve arabica coffee beverage quality. The results showed that *W. anomalus* UV22-3 as a starter culture for coffee fermentation could enhance the coffee flavor quality compared to the control experiment (without inoculum). Fermented arabica coffee by strain UV22-3 showed a higher cupping score than wild type and a control condition with unique cupping notes. According to the flavor profile evaluated by Q-graders, the result of this sensory evaluation is 82. Microbial population in the fermentation broth was evaluated. The total yeast number was stable, while the total bacteria was higher after 24 h of strain UV22-3 fermentation. The pH value slightly decreased when the total dissolved solid increased. This research is one alternative to improve the quality of coffee in Thailand by using a novel yeast strain.

Keywords: Coffee, Fermentation, Wet Process, Starter Culture, Yeast, Mutagenesis, Ultraviolet irradiation

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[1] Department of Microbiology, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand
[2] Department of Science and Bioinnovation, Faculty of Liberal arts and Science, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand
[3] Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand
[4] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani 12120, Thailand
[*] Corresponding author, email: sukhumaporn@g.swu.ac.th

Introduction

Coffee is one of the most popular and appreciated non-alcoholic beverages worldwide. "The Coffee Belt" is an area suitable for growing coffee along the equator with cultivation in Brazil, Vietnam, Colombia, Indonesia, Ethiopia, Honduras, Guatemala, and Thailand [1]. The Arabica coffee of Thailand is grown in the northern part such as Chiang Mai, Chiang Rai, Mae Hong Son, etc. The four main factors affecting coffee quality are variety, terroir, coffee processing, and brewing [2]. Thai farmers have various methods to process their coffee cherry, and the wet process is typically used to make consistent quality at the coffee farm in every year [3]. The mucilage layer inside cherry coffee comprises of polysaccharides (pectin), sugar, cellulose, starch, and many nutrients suitable for developing microorganisms such as yeast, bacteria, and fungi [4, 5]. These microbes play essential roles in degrading mucilage by producing various enzymes, alcohols, and acids during fermentation that influence the final beverage [6].

Wickerhamomyces anomalus has been reported and published on pectinolytic yeast from the coffee process and starter culture production for coffee fermentation [7]. *W. anomalus* YWP1-3 demonstrated high pectinase activity and grew very well in mucilage broth. Moreover, it showed high efficiency as a starter culture for Thai Arabica coffee fermentation [7]. *W. anomalus* is deemed harmless for health and the environment, and can grow under severe environmental stress [1].

The previous report showed that some of mold, treated with UV, increased pectinase production [8]. Industrial microorganisms selected natural and genetic modification microorganisms by various techniques for better performance, such as UV irradiation and chemical mutagenesis.

Therefore, our previous work expected to improve *W. anomalus* strain by induced mutation using ultraviolet (UV) radiations and chemical mutagenesis, and tried to use it as a starter culture in a controlled coffee fermentation. Strain UV22-3 showed high pectinase production, good growth in mucilage broth, and better carbon utilization ability. This study aims to utilize the *Wickerhamomyces anomalus* strain UV 22-3 as a starter culture for Thai arabica coffee fermentation to improve arabica coffee beverage quality.

Materials and methods

Yeast strains

Wickerhamomyces anomalus UV22-3 was mutated by UV irradiation. *W. anomalus* YWP1-3 is wild type used as a control strain in this experiment. The stock culture was stored at -20 °C, and was reactivated in YM broth (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose, and pH adjusted to 6.0).

Starter culture preparation

Wild type and strain UV22-3 were used as a starter culture for the coffee fermentation. Each strain was prepared from fresh culture to pre-culture in 50 mL of YM broth and incubated at room temperature for 24 h at 150 rpm. Five milliliters of the culture broth was transferred to 50 mL YM broth and incubated at room temperature for 48 h at shanking speed of 150 rpm. After centrifugation

at 10,000 rpm for 10 min, the cell pellet of the mutant strain was used for the starter culture of the coffee fermentation process.

Arabica coffee fermentation

All starter cultures of each strain were mixed (10% inoculum) with 10 L water and 7 kg cleaned and freshly de-pulped coffee beans in the tank. De-pulped coffee beans were incubated at an ambient temperature of around 13-25 °C for 48 h at Community Enterprise Groups in Thep Sadet Sub-district, Doi Saket District, Chiang Mai Province. After 48 h of fermentation, the coffee beans were washed several times to get the cleanliness of the coffee. Control fermentation was prepared using the above method without inoculating the starter culture. The fermentation broth was collected and used for further analysis during coffee fermentation.

Sensory analysis

Green coffee beans were roasted at 115.6 °C until the Agtron gourmet color scale was 55-65 Agtron. Cupping sensory testing within 24 h after roasting followed SCAA Protocols (Cupping Specialty Coffee) [9]. The Cupping Form records essential flavor attributes for coffee: fragrance/aroma, flavor, aftertaste, acidity, body, balance, uniformity, clean cup, sweetness, defects, and overall. The specific flavor attributes are positive scores of qualities reflecting a judgment rating by the cupper. Defects are negative scores denoting unpleasant flavor sensations. The overall score was based on the flavor experience of the individual cupper as a personal appraisal.

Sample collection, microbiological analysis, pH and Brix measurement

Microbial counts and physical parameters were performed randomly in the liquid component of samples in triplicate (before, 0 h) and after (48 h) of fermentation. A portable pH meter, refractometer, and TDS meter were used to monitor the fermenting mass's pH, Brix, and TDS values at each sampling point. The microbial growth of the fermentation process was determined. The 10-fold serial dilution was made by diluting 0.5 mL of sample with 4.5 mL of normal saline. Further 10-fold serial dilutions, ranging from 10^{-2} to 10^{-4}, were prepared, and the microbial counts were determined according to the standard spread plate method. Total viable bacterial counts were measured using Plate count agar (PCA) and incubated at room temperature for 24 h. The counts of yeasts were determined using YM agar and incubated at room temperature for 24 h. The number of lactic acid bacteria (LAB) was analyzed using de Man Rogosa Sharpe (MRS) agar and incubated at room temperature for 48 h under anaerobic conditions using an anaerobic jar and gas pack. The number of colony-forming units (CFU/mL) was recorded after incubation.

Statistical analysis

This study used the statistical program IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y. USA) for statistical analyses. The significant level was in a two-sided *p*-value

≤ 0.05. Average and standard deviations of evaluation data were analyzed by t-Test and one-way ANOVA followed by posthoc comparison of means by LSD test.

Results and Discussion

Viable count of microorganisms in coffee fermentation

Yeast count was determined from fermentation broth samples. The results showed that yeast number was constant from 0-48 h of fermentation (Figure 1A). It might be due to the heavy inoculum of the starter culture in the fermentation tank at 0 h, and yeast still adapts to the fermentation environment and produce some essential chemical compounds that affect the flavor quality of arabica coffee. In contrast to this work, the number of pectinolytic yeasts, *S. cerevisiae* KNU18Y13 increased to 8.23 log CFU/mL after 48 h of coffee fermentation during wet processing [1]. Moreover, the yeast population in the control experiment was not significantly different from the experiments that inoculated with *W. anomalus* UV22-3 because other natural yeast strains in the fermentation process could grow very well. Still, this yeasts consortium was unable to improve coffee quality.

The viable count of total bacteria was analyzed at 0 and 48 h, as shown in Figure 1B. The bacterial number was increased after coffee fermentation by wild type and UV22-3. This result indicated that inoculation of starter culture affected suitable conditions for bacterial growth, such as pH value and oxygen concentration. Moreover, UV22-3 could degrade mucilage pectin and created a sugar compound that bacteria can use for growth. In a previous study, microbial ecology in Australian coffee fermentation showed that the initial total aerobic mesophilic bacterial (AMB) population was increased from 5 log CFU/g to 7.2 log CFU/g by the end of the fermentation [10]. In contrast to Mahingsapun et al. [11] report, total bacterial counts decreased by approximately 1 log CFU/mL in most fermentation conditions using microbial cocktails under incubation at 25 °C.

The lactic acid bacterial number was studied during the wet fermentation process. Interestingly, LAB decreased in coffee fermentation conditions that contained starter culture (Figure 1C). Yeast starters might produce some metabolites, making the fermentation environment unsuitable, and affected LAB growth. This phenomenon is suitable for coffee flavor quality. Lactic acid bacteria are often found in coffee fermentation because LAB produces many acids, such as lactic acid, acetic acid, propionic acid, and others [11]. The presence of these acids impacts coffee beverage quality. A high concentration of acetic acid (over 2 g/L) causes an undesirable vinegary taste [12, 13]. Furthermore, propionic and butanoic acids might affect an unpleasant flavor in roasted coffee beans [14, 15].

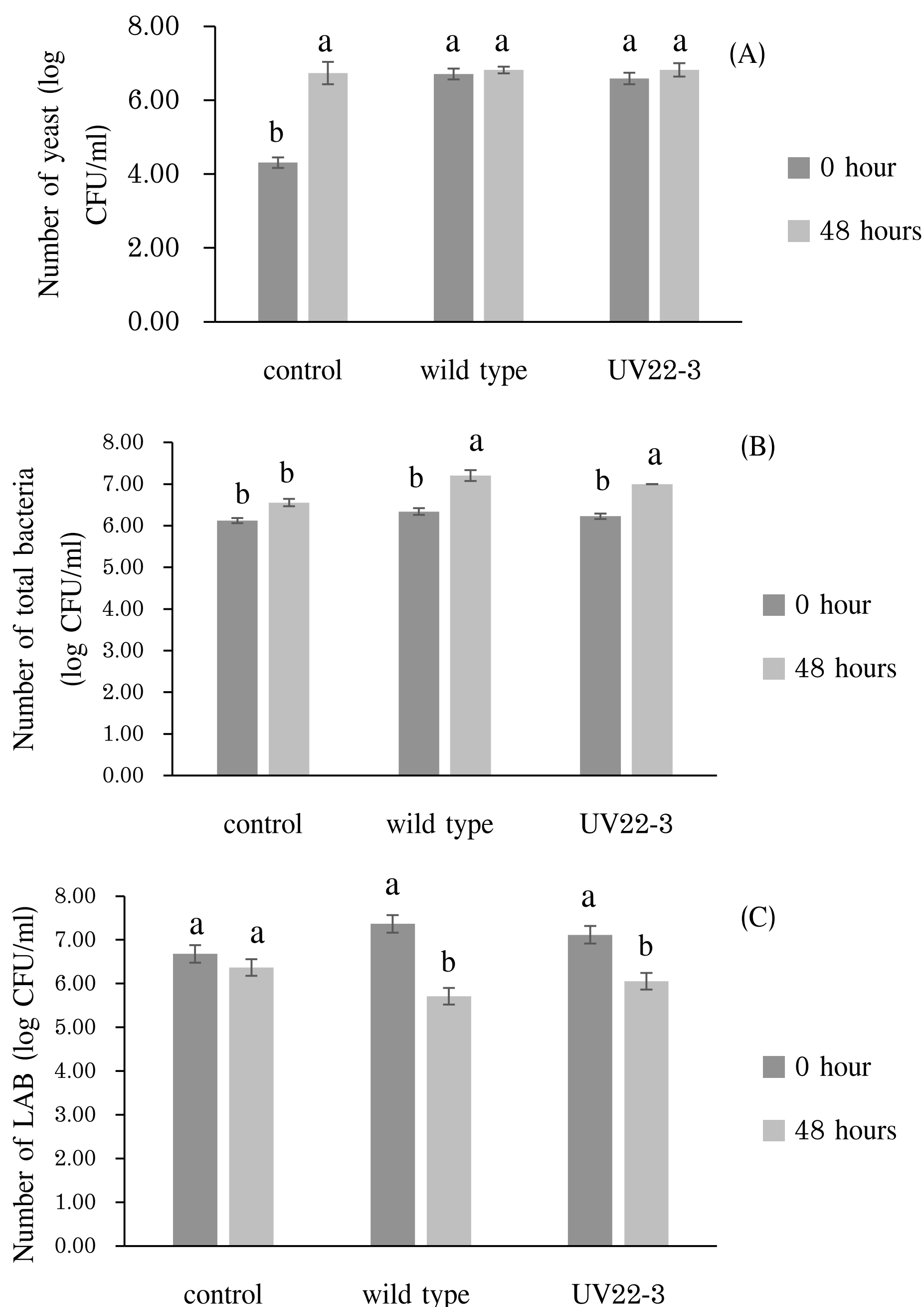

Figure 1 Microbial count, yeast (A), total bacteria (B), and lactic acid bacteria (C) in coffee fermentation broth derived from the wet process at 0 and 48 h of incubation. The value log CFU/mL represents the mean values of triplicate (n = 3), with error bars showing the standard deviation of the mean. Different letters (a–b) indicate statistical differences among different samples ($P < 0.05$).

pH, Total dissolved solid and Brix measurement

pH value during coffee fermentation was evaluated as the fermentation progressed. A decrease in pH was achieved in all conditions (Table 1). However, in fermentation containing starter culture, the pH value slightly decreased. This is due to the decreased LAB number in these conditions, as shown in

the previous section. It is commonly known that the minimum pH value occurring during coffee fermentation is primarily responsible for acid accumulation in the coffee process [16]. In addition to the action of lactic acid bacteria, the decreased pH may also be caused by producing certain acids from yeasts, such as citric, lactic, α-ketoglutaric, pyruvic, and isocitric acids [17-19]. Total dissolved solid (TDS) and °Brix were recorded. All conditions showed higher values after 48 h of coffee fermentation. This phenomenon comes from the microbial community's effect of pectin degradation during fermentation. In addition, it can be seen from the experimental results that the change in TDS value in the control experiment was higher than that of the other experiments due to 0 h of fermentation wild type and UV22-3 strains being heavily inoculated (10% inoculum). These inoculums caused the initial TDS value to be higher than the control experiment, resulting in less change in TDS. Sugar concentration collected from the product of mucilage degradation affects the higher value of °Brix. Almost two folds of °Brix value increased in coffee fermentation by UV22-3 due to this UV mutant strain's high efficiency of pectinase activity production.

Table 1 pH, Total dissolved solid (TDS) and °Brix of coffee fermentation broth in various conditions.

Fermentation conditions	**pH**		**Total dissolved solid (TDS)**			**°Brix**	
	0 h	**48 h**	**0 h**	**48 h**	**△TDS**	**0 h**	**48 h**
control (without starter)	4.90±0.20[a]	4.33±0.58[b]	118.70±6.80[a]	314.30±50.52[b]	195.60[a]	0.33±0.58[a]	0.57±0.15[b]
wild type	4.67±0.58[a]	4.40±0.00[b]	198.30±71.65[a]	323.67±76.79[b]	125.37[b]	0.47±0.21[a]	0.60±0.26[b]
UV 22-3	4.73±0.58[a]	4.50±0.58[b]	238.30±30.75[a]	360.30±43.66[b]	122.00[b]	0.53±0.11[a]	0.93±0.21[b]

This table demonstrates mean±SD of triplicate. Different letters (a–b) indicate statistical differences between 0 and 48 h of samples (P < 0.05) by t-Test analysis.

Evaluation of fermented arabica coffee

This report demonstrates the development of a wet fermentation process using starter cultures derived from UV mutation. Table 2 summarizes the sensory testing and cupping notes of arabica coffee beverages improved as a result of the work. Fragrance/aroma, flavor, acidity, body, uniformity, balance, sweetness, clean cup, or overall were evaluated by Q-arabica graders. Inoculated fermentations with UV22-3 produced arabica coffee beverages with a prominent cupping note, as shown in Table 1, resulting in a higher score than the uninoculated and wild type condition. This result suggested that the mutant strain, UV22-3, contributed unique flavor to the coffee products. According to cupping notes, fermented coffee by UV22-3 demonstrated high sweetness, medium acidity, high body, and long aftertaste. It might be due to strain UV22-3 showing high pectinase production can liberate high sugar content in coffee beans, and predominant in mucilage broth, and better carbon utilization ability affects lower lactic acid bacteria during fermentation [7].

Many reports showed that inoculation of starters in coffee fermentation enhances Thai arabica coffee flavor quality [3, 7, 20]. However, no reports on coffee fermentation using a novel strain as a mutant by UV irradiation have been observed.

Table 2 Sensory analysis and the cupping score of coffee beverages derived from various fermentation processes.

Fermentation condition	Cupping score	Cupping notes
Control (without starter)	75.50±2.50	caramel, vanilla, bell pepper, green vegetable, hay, nutty, peanut, dark green (low sweetness, flat acidity, short aftertaste, less body, very bitter)
Wild type	80.25±1.65	floral, vanilla, caramel, malt, cocoa, prune, honey, chocolate, red apple, yellow flower, white flower (medium sweetness, low acidity, medium body, long aftertaste)
UV22-3	82.00±2.14	nutty, pineapple, caramel, honey, cocoa, lemon (high sweetness, medium acidity, high body, long aftertaste)

Conclusions

Post-harvest processing of arabica coffee is one of the critical processes involved in coffee quality. We succeed in manipulating yeast strain by using UV irradiation. *Wickerhamomyces anomalus* UV22-3 is suitable as a starter culture in arabica coffee fermentation. The microbial community in coffee fermentation was changed, such as a lower number of lactic acid bacteria and a higher bacterial population. After fermentation, it showed a higher cupping score (82.00) than the wild type (80.25) and special cupping notes. Therefore, this is the first report to develop a mutant yeast strain as inoculum for coffee fermentation under a wet process and the results demonstrated higher coffee flavor quality.

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