Survival of *Candida tropicalis* and *Lactobacillus plantarum* starter culture after using protective agent and drying

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**Abstract**

Yeast *Candida tropicalis* and lactic acid bacteria *Lactobacillus plantarum* were isolated from Ka-nom Tuay-fu as traditional starter culture in Thailand. The isolates were used on labolaratory-scale process. Effect of temperature drying (40, 50 and 60ºC) on the survival and activity of *C. tropicalis* and *L. plantarum* were investigated. Rice flour was used as supporting material. The temperature drying had affect cell viability, moisture content, water activity and drying time. The cell viability of starter cultures have significantly affect under different temperature. The dry condition at 40ºC for 16 hr was obtained the high cell viability of *C. tropicalis* and *L. plantarum* with 7x10^6 and 5x10^6 CFU/5g, respectively. The starter culture powder can dehydrated to low of the moisture content at 10% and water activity 0.4. Sucrose and glucose solution were used as protective agent at concentrations of 0-15%(w/v). Glucose 15%(w/v) showed superior protective on recovery cell survival of *C. tropicalis* and *L. plantarum* up to 99 and 90%, respectively. The result revealed the optimal condition of drying process can be used for preparation of the Ka-nom Tuay-fu starter culture.

**Keywords**

*Candida tropicalis*

*Lactobacillus plantarum*

Starter culture

Protective agents

Drying

**Introduction**

Thai steam bread or a fermented spongy rice cake is one of the traditional fermented food and a few maker in Thailand as Ka-nom Tuay-fu. The production is still homemade. It is made from rice flour, palm sap or palm sugar, water and Loog-pang Khaomak which are major ingredients. Loog-pang Khaomak is a traditionally mixed starter culture with variety of microorganisms whose strains have the highest growth rate and predominant activities during fermentation. A significant disadvantage in use Loog-pang Khaomak for Ka-nom Tuay-fu is difficultly control the quality of products. All time processes was about 2 days. So far, the baking powder or baker’s yeast had been used and obtained the spongy texture, but a specific flavor-acidity flavor from lactic acid bacteria has not attained yet. The process of Ka-nom Tuay-fu was include to crush the rice, mix the ingredients, ferment with natural inoculum and steam. The steam bread products was a sweet with a little sour taste and good smell. It used as a popular dessert in the south of Thailand. Our research has been isolate and achieve the new cultures have important adventages, such as a specific flavor and a spongy texture. It has been selected a proper strains with good ability and preparation of pure inoculum for steam bread, mainly yeast *Candida tropicalis* and lactic acid bacteria *Lactobacillus plantarum* (Maneesri and Masniyom, 2015). Both strains were studied the optimum of freeze drying process and can used for starter culture preparation. Rice flour was used as supporting material. Moreover, sucrose and glucose solution were used as protective agent. Freeze drying process has effect on cell viability. But the cost is higher than other drying methods (Liming et al., 2014). In the other fermentation food, “sourdough bread” is well know that using the mixed starter culture containing lactic acid bacteria (LAB) and yeasts (Caballero et al., 1995; Gobbetti et al., 1995; Linko et al., 1997; Meignen et al., 2001). The pure cultures expose a remarkable improvement such as fermentation time reduction and desirably sensorial quality. The wet pure culture is incompatible with the commercial one and unfavorably applied in fermentation and storage. Therefore, desiccation has been the preferable method to prepare the starter cultures has to reach the targets as viability, vitality, storage stability and handling. (Santivarangkna and Foerst, 2015)

In order to minimize damage during drying, protective agents are added to the cell suspension before drying and has the proposed: preferential hydration, water replacement and glass transition.
(Santivarangkna et al., 2008). Protective agents have been used in several researches such as triols, polyalcohols, monosaccharide, disaccharide and polysaccharide (Hubalek, 2003). The type and concentration of protective agent depends mainly on the microorganism. The survival of low temperature drying methods of L. plantarum CIF17AN2 as showed at 45°C. The survival of freeze dried C. sake cells was increased to 30-40% by using 5% or 10% glucose or 10% sucrose (Abadías and et al., 2001). Glucose has been used to improve the survival cells at concentration 1-18% and 1-68% for sucrose (Hubalek, 2003). It is well know that drying process is the common technology and reduce cost. However, little information regarding the optimal process in Ka-nom Tuay-fu starter powder has been reported. Therefore, it is necessary to investigate the viability of C. tropicalis and L. plantarum starter culture in the different temperatures and protective agents during drying.

Materials and Methods

Microorganism

C. tropicalis TISTR 5922 and L. plantarum TISTR 2083 were isolated from Loog-pang Khaomak used as Ka-nom Tuay-fu production that it were obtained from Pattani province, Thailand (Maneesri and Masniyom, 2012). Both strains were collected at Thailand Institute of Scientific and Technological Research (TISTR), sub-cultured every month on Yeast Peptone Dextrose (YPD) agar and Man Rogosa and Sharpe (MRS) agar, respectively and stored at 4°C until used.

Cell preparation

C. tropicalis and L. plantarum were respectively cultured in 500 ml flask containing 300 ml of YPD broth and MRS broth. The culture conditions to obtain the logarithmic phase were 30°C, 110 rpm and 12 hr for C. tropicalis and 21 hr for L. plantarum. Cells were harvested by centrifugation at 9,000 xg for 10 min at 4°C. The supernatant was decanted and the cell paste was resuspended in 30 ml distilled water. This process was twice repeated to collect the cell for subsequent mixing with distilled water or protective agents.

Protective agent

D-glucose and sucrose were two types of protective agent and distilled water was used as a control treatment for comparison. Protective agents were prepared at 5, 10 and 15% (w/v) concentrations and subsequently autoclaved at 121°C for 15 min before use.

Sample preparation for experiments

Cell paste obtained as described above was resuspended in distilled water. Cell suspension of C. tropicalis and L. plantarum were contained approximately 10^6-10^7 CFU/ml and 10^7-10^9 CFU/ml, respectively. The suspension (5 ml) was transferred and mixed with 5 g sterilized flour in petri dish to make the starter culture. Starter culture was dried by hot air oven at 40°C, 50°C and 60°C, the sample was tested every 4 hour for 24 hr.

The cell viability of both treatments were determined before and after drying by pour plate method. The percentage of cell viability was expressed as ratio between the total number of viable cells after drying and total number of viable cells before drying, which subsequently multiplies with one hundred.

Storage experiments

Starter culture powder of C. tropicalis and L. plantarum were filled about 5 g in plastic bag (NY/LLDPE size 90x75 mm.), aluminium foil (ALU/LLDPE size 90x75 mm) with vacuum or non vacuum with stored at 4°C and room temperature kept at 30°C. The sampling was done every 15 days up to 90 days storage. At every sampling point was measured the cell viability, water activity and moisture content. The percentage of survival was calculated from the viable cell counts before and after storages at each sampling time.

Determination of water activity and moisture content

Moisture content was determined according to the method of AOAC (2000) and water activity measured with water activity meter (Aqua Lab, 4TE).

Statistical analysis

The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan’s multiple range test (DMRT) at a 5% probability level (p ≤0.05). All analyses were run in triplicate.

Results and Discussion

Effect of temperature drying

Drying condition is related to the temperature and time drying on the cell survival which leads to an economic. The temperatures were varied to investigate their effects on the survival cells of C. tropicalis and L. plantarum starter culture. After
drying, the viability of *C. tropicalis* and *L. plantarum* were drastically decreased and no cells at 60°C for 4 hr (Figure 1). The highest viability of *C. tropicalis* starter culture was achieved under drying operated at 40°C. The total count of cell from culture was decreased only 1 log CFU/g at 40°C for 12 hr and kept stable until 24 hr. Whileas, drying at 50°C for 4 hr found that the cell count of cell culture was slightly decreased 3 log CFU/g and no cell viability. The viability of *L. plantarum* starter culture was decreased 1.95 log CFU/g with drying at 40°C for 16 hr. The temperature drying at 50°C and 60°C were obtained the cell viability of *L. plantarum* starter culture similar to *C. tropicalis*. The moisture content of *C. tropicalis* and *L. plantarum* starter culture after drying at 40°C for 16 hr were 10.4%, respectively. The results showed that each temperatures were effect difference in cell viability and the moisture content. The water activity was related to the moisture content and the moisture content is an important parameter for cell viability. For the samples dried at 40°C the water activity was 0.4. The high cell viability, sufficient moisture content and water activity were important in the stability of dried starter cultures. In our results, drying at 40°C for 16 hr could suggested as optimum for cell starter culture and chosen to carry out further experiment. In addition, rice flour was used as carrier material might protect the cell of *C. tropicalis* and *L. plantarum* during longer drying process. This conditions were chosen as the most effective and economical method. Meuser et al. (1995) noted about the microbial activity of dried sourdough on the first at 40°C and reduce to 30°C that used as starters for sourdough fermentation. According to Hongpattarakere and Uraipan (2015), resistant starch from unripe saba banana was effectively protected *Lactobacillus plantarum* CIF17AN2 during drying process. Moreover, the survival of microorganisms have many factors, such strains (Bauer et al., 2012), temperature of drying (Fu and Chen, 2011) and protective agents (Hubalek, 2003).

**Effect of protective agents**

The effects of different protective agents and their concentrations on cell viability of *C. tropicalis* and *L. plantarum* starter cultures during drying process were investigated. Results are shown in Figure 2. The viability of *C. tropicalis* was increasing corresponding to the increase in concentrations of glucose and sucrose from 5% to 15%. The 10% and 15% glucose concentrations were effectively preserve the viability of *C. tropicalis* from 6.21 logCFU to 6.78 and 7.12 logCFU, respectively. The cell survival of *C. tropicalis* was 95 and 99%, respectively (Figure 3). When using 10% and 15% sucrose of protective agent showed the cell survival 95 and 94%, respectively. Whereas, the viability of *L. plantarum* starter culture with 15% glucose observed increase the viability from 6.02 logCFU/g to 7.03 logCFU/g.
logCFU/g and the cell survival was 90%. The viability of the cell was low when using 10% glucose. Glucose and sucrose at 5% were no increasing in cell viability. Therefore, glucose at 15% concentration showed the highest cell survival up to 90%. As the result, it could be concluded that glucose greatly enhanced the retention of viability of C. tropicalis and L. plantarum starter cultures during drying. The moisture content of C. tropicalis and L. plantarum starter culture after drying were slightly decreased. But the effect of glucose concentration was increased the water activity (Figure 4). At the highest survival obtained at 15% glucose concentration for L. plantarum starter culture, moisture content were 8.39% and water activity 0.4. Results of glucose as the protective agent and addition to the cell suspension before drying has been reported that sugars are used in preparation of dried starter cultures for fermented food. When drying to a slowly low moisture content and leads to a lower survival rate (Santivarangkna et al., 2007; Santivarangkna et al., 2008). Interestingly, dried starter cultures at low temperature drying is higher viability than freeze-dried starter. For freeze drying, the cell survival of C. tropicalis and L. plantarum are 73 and 92%, respectively (data not shown).

Survival of C. tropicalis and L. plantarum
Survival of C. tropicalis and L. plantarum after storage in each conditions at different package as aluminium foil (A), plastic bag (N), vacuum (V) or non-vaccine sealing (nV), temperatures (4, 30 °C) by count cell viability. The slight viability loss of C. tropicalis

Figure 3. Cell survival of C. tropicalis (a) and L. plantarum (b) starter with a protectant agent as glucose and sucrose during drying at 40 °C for 16 hr

Figure 4. Moisture content and water activity (aw) of C. tropicalis (a) and L. plantarum (b) starter with a protectant agent as glucose and sucrose during drying at 40 °C for 16 hr
and _L. plantarum_ starter powder were observed from the beginning to 90 days of storage (Figure 5).

At 4°C storage, the starter powder product in an aluminium foil sachet and plastic bag were higher cell viability than that stored at the room temperature. Cell survival of _C. tropicalis_ under storage under treatment AV4, ANV4, NV4 and NNv4 showed closely and higher than 95%. Cell survival of _L. plantarum_ was the highest at 90% in AV4 and following to NNv4, ANv4 and NV4 as 89, 88 and 75%, respectively. At room temperature, a sharp decrease of viable cells were detected at both package within 45 days. Our result showed the temperature of storage was effective for protecting cells. It can be concluded that storage at low temperature is required to maintain viability of culture starter powder. The water activity and moisture content were slightly increase and not effective to survival of _C. tropicalis_. But the survival of _L. plantarum_ cells were a few reduced during in 90 days.

**Conclusion**

Drying were affected on _C. tropicalis_ and _L. plantarum_ starter culture, with decreased viability of cell during the drying process. Cell can be damage due to thermal and dehydration stress. This study demonstrated the possibility of addition of glucose as protective agent were improved the survival depend on concentration. In optimization condition of drying can be use 40°C for 16 hr. and the better results in cell viability, drying time, even comparable cost to preparation of _C. tropicalis_ and _L. plantarum_ starter culture for production of Ka-nom Tuay-fu. In this work, it was shown that the starter culture powder for Ka-nom Tuay-fu can be use easily and increase the consuming in the home. Therefore, to application for production of Ka-nom Tuay-fu and can use for future studies to development the product.

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