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ISOLATION OF SOME YEAST STRAINS FROM NATURAL SOURCES HAS A POWERFUL PROGRAMMING APPLICATION FOR BREAK PRODUCTION

Luu Hong Son[1], Pham Thi Phuong[1], Vi Đại Lâm[1], ,Nguyen Thi Quynh Huong[1], Bui Thi Kim Oanh[1], Nguyen Thi Mai Thuy[1], Pham Thi Vinh[1], Nguyen Thi Tinh[1], Igbonekwu-udoji Reagan Jonas[1], Pham Phuong Thu[2], Dinh Thi Kim Hoa[1]

1TNU - University of Agriculture and Forestry,Viet Nam

2Hanoi Pedagogical University2, Viet Nam

Email address: luuhongson@tuaf.edu.vn

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Abstract:

Research on isolating yeast in bananas, mangoes, oranges, litchi, plums, pineapples, peaches, tangerines, apples and guava, which were harvested in Thai Nguyen city. Successfully isolated 01 yeast strain obtained from banana for application in bread production. The possibility of bread production was tested using a yeast strain obtained with good sensory quality..

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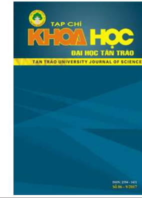

PHÂN LẬP MỘT SỐ CHỦNG NẤM MEN TỪ MỘT SỐ LOẠI QUẢ ĐỂ SẢN XUẤT BÁNH MÌ

Lưu Hồng Sơn[1], Phạm Thị Phương[1], Vi Đại Lâm[1], Nguyễn Thị Quỳnh Hương[1], Bùi Thị Kim Oanh[1], Nguyễn Thị Mai Thuỳ[1], Phạm Thị Vinh[1], Nguyễn Thị Tình[1], Igbonekwu-udoji Reagan Jonas[1], Đinh Thị Kim Hoa[1], Phạm Phương Thu[2]

1Trường Đại học Nông Lâm - ĐH Thái Nguyên,Việt Nam

2Trường Đại học sư phạm Hà Nội2, Việt Nam

Địa chỉ email: luuhongson@tuaf.edu.vn

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Từ khóa:

nấm men;tự nhiên; bánh mỳ.

Tóm tắt

Từ 10 lọai hoa quả trong tự nhiên nhóm nghiên cứu đã phân lập thành công 01 chủng nấm men thu nhận từ chuối để ứng dụng trong sản xuất bánh mì. Đã thử nghiệm khả năng sản xuất bánh mì bằng chủng nấm men thu nhận được cho chất lượng cảm quan tốt.

1. Introduction

The current natural source of yeast that is said to have good quality is in oranges, tangerines, and bagasse, ... The yeasts on the fruit surface are usually capable of fermenting many sugars and are tolerant of high alcohol concentrations. In food technology, yeast has many applications, especially in fermented products such as wine, beer, bread, kimchi, vegetable juice. In the production of bread, yeast contributes to the nutritional value, taste, and the amount of gas released that increases the size of the dough, contributing to the shape, size, consistency of the dough, and attractiveness of the cake [3]. Nowadays, there are many forms of commercial yeast like dry yeast, fresh yeast, and instant yeast. These products are still being studied and improved every year to increase quality and convenience for customers. In the face of consumer demand for bread, continuous improvements in the bread production proCess to increase the flavor of the bread are necessary. Different yeast strains are continuously isolated and tested to apply to production practice is a potential direction.

2. Materials and methods

2.1. Materials

Yeast strains derived from nature have a strong reproduction ability for application in bread making.

Materials: Straight inoCulation rod, hook inoCulation rod, piercing rod, triangle implant, tip, micropipette, pipette, test tube, falcon tube, petri dish, conical flask, measuring cup, measuring tube, cuvette tube, cell counting chamber, lamella, microscope slide, cloth towel, cleaning paper, plastic bag, belt, fruit knife, memo pad, marker pen.

Equipment: Analytical balance, microwave oven, incubator, spectrophotometer, centrifuge, oven, sterilizer, microscope, refrigerator, shaking incubator.

- Chemicals: Distilled water, filtered water, methylene blue, alcohol 96°, alcohol 70°, flour, fat flour, salt, bread additives, market yeast.

- YEPD medium:

Ingredients:	g/L	Sterilize medium
Agar	20	AutoClave at 121°C, pressure 1 atmosphere, time 60 minutes
Peptone	5	
Yeast extract	3	
Glucose	20	
Chloramphenicol antibiotic	0.4%	

2.2. Methods

Collecting 10 samples including Banana, mango, orange, litchi, plum, pineapple, peach, tangerine, apple, guava, naturally collected in Thai Nguyen city, preserved at the Food Technology Laboratory, Faculty of Biotechnology & Food Technology, Thai Nguyen University Agriculture and Forestry. Samples were stored at 4°C for about 3 days.

Experiment 1: **Isolation of yeast**

Proliferative culture

Samples of fruits such as banana, mango, orange, lychee, plum, pineapple, peach, tangerine, apple, guava were sliced, then put five grams each fruit type into two test tubes containing 10 mls sterilized liquid YEPD medium, then keep the test tubes for 2 days to create favorable condition for yeast growth.

Dilution and inoCulation (spread)

After 2 - 3 days, the culture solution to the fruit sample was diluted in series at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. Each dilution was inoCulated onto petri dishes supplemented with 0.4% chloramepheniCol. Use a micropipette to aspirate 100 µls of diluent of each concentration on the surface of YEPD agar in a petri dish. Use a triangular inoCulation rod to equilibrate the diluent on the agar surface until the surface of the medium is dry. Culture at room temperature, followed after 12-24 hours until colony formation [3].

Streak plate technical

In microbiology, streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. The dilution or isolation by streaking method was first developed by Loeffler and Gaffky in KoCh's laboratory, which involves the dilution of bacteria by systematically streaking them over the exterior of the agar in a Petri dish to obtain isolated colonies which will then grow into the number of cells or isolated colonies. The decrease of bacteria should show that colonies are sufficiently spread apart to effect the separation of the different types of microbes.

The streaking is done using a sterile tool, such as a cotton swab or commonly an inoCulation loop. The inoCulation loop is first sterilized by passing it through a flame. When the loop is cool, it is dipped into a petri dish containing isolated colonies then dragged across the surface of the agar in 3 different lines. The loop then is re-sterilized to make another once. Culture petri dishes at room temperature, monitor after 12-24 hours until colonies form.

Morphological observation

Each microbial colony was cultured in YEPD medium mixed in a drop of sterile distilled water on a slide, covered with a slide, and observed under an optical microscope at objective 40.

Count yeast cells by using a hemoCytometer

Use a micropipette to take a drop of the sample to determine the cell concentration, put it in the gap between the counting chamber and the lamella. The number of yeast cells under the microscope should be counted; at least 4 large plots should be repeated. If the cell concentration is too large (more than 200 cells in 1 large cell), proCeed with dilution. Take care to prevent yeast from settling during sampling. Depending on the number of cells, it is possible to count all the cells in the cell or only the cells contained in a representative large number of squares. Count in a zigzag from left to right, top to bottom. Count all yeast cells inside of 16 cells, yeast cells on the outside edge of 16 cells count only yeast cells on the top and left edges (and only yeast cells on the right side). in at least 1/2) [3].

Preserving and keeping seeds

From the whiskers, the best growth specimens will be removed and transferred to a slanted agar containing YEPD medium with cotton cork. Next, these samples will be cultured in an incubator for about 24 hours to continue to multiply. Finally, store at 4°C.

Experiment 2**: Evaluation of the effect of incubation time on the growth of yeast**

This proCess was carried out by determining the optical density/absorbance (OD) of the yeast species using spectrophotometer (Genova). The absorbance of the cultures was determined at 1 hour intervals of 24 hours starting at time zero (0 hour). The wavelength of the spectrophotometer was set to 600 nm and blanked with a cuvette containing 2 mL sterile YEPD medium. The culture (1 mL) was pipetted into a fresh cuvette and diluted with 1 mL sterile YEPD medium. The cell suspension cuvette was placed into the cuvette slot and the cuvette chamber was closed. The absorbance of the cell suspension was recorded

and the proCess was repeated at an hour interval of 24 hours. The optical densities for cell suspensions with absorbance greater than 1 were calculated using Eqn (2). Optical density (absorbance) = 2 – log %T (2) where %T is percent transmittance [5].

Experiment 3: Evaluation of the influence of shaking culture time on the growth of yeast

The isolated yeast strain was grown in liquid YEPD medium, shaken at 180 rpm, at room temperature. From 3 hours after implantation, measure OD every 1 hour. The increase in the OD value is the basis of assessing the increase in the concentration of yeast in the solution.

To test the appropriate shaking culture time, after 24 hours of culture in the liquid medium, the yeast strain entered the logarithmic growth phase. Collect 300 ml of shaking culture medium, centrifuge to remove the antibiotic solution, add distilled water and test dough. Dough size was assessed after 2 hours of incubation.

Experiment 4: Test the ability to produce gas to increase dough volume of yeast strains obtained from fruits

Yeast strains obtained from bananas were shaken in 500 ml YEPD medium, 180 rpm, room temperature. After 2 days of culture, use the test biomass as the bread dough, replacing the commercial yeast strain. Evaluation of yeast strain's ability to make dough at intervals of 1 hour to 6 hours.

Experiment 5: Evaluation of the effect of additive content in bread production

To evaluate the effect of bread additive content on dough, baking ability, flour bloCks were added with additives at the following concentrations: 0%; 1%; 5%; 15%; 20%. Monitor the volume of the dough over time.

Experiment 6: Evaluation of the effect of baking proCess on bread quality

To evaluate the influence of the baking proCess on the quality of bread, two types of equipment are used: SANAKY mini oven and industrial toaster oven. Two test cases with same conditions, same proCessing, mixing recipe and same operator. Product rating.

2.3. Statistical analysis methods

Data were analyzed by one-way analysis of variance (ANOVA) and Fisher's PLSD post-test at P ≤ 0.05 using SPSS software (version 20).

3. Results and discussion

3.1. Isolation from yeast from natural sources of strong proliferative ability and application in bread production

3.1.1. Yeast isolation

Samples of fruit such as banana, mango, orange, lychee, plum, pineapple, peach, tangerine, apple, guava were sliced, and cultured in YEPD medium for 2 days, creating favorable conditions for microorganisms to grow. Carry out dilution, inoCulation, and streak culture on antibiotic-containing media to isolate yeast. As a result, 05 strains of microorganisms were obtained, in which the strain isolated from the previous steps had the fastest growth rate. Formation of colonies visible to the naked eye after 12 hs of culture (Figures 1A, 1B). Colony morphology is spherical, milky white or clear white, smooth margin, capable of growing on nutrient-poor media containing only 5% cane sugar and agar (Figure 1C). When viewed under a microscope, strain cells isolated from previous steps are ovoid or oval in shape, with budding. These are the characteristics that fit the yeast groups *Saccharomyces cerevisiae* (Figure 1D). So strains isolated from previous steps were selected for sequencing to identify species, keep and use for further experiments. Storage conditions in test tubes containing YEPD medium, at 4°C.

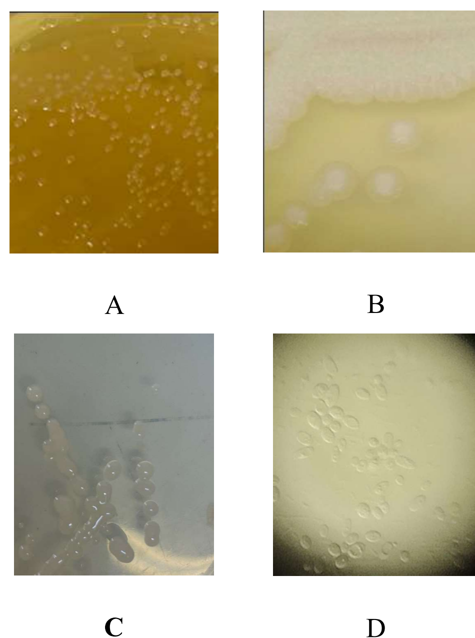

Figure 1: Yeast strains from some natural fruits

A- InoCulation results of the isolates obtained from the previous steps

B- Clean mustache transplant results

C- Bacterial strains isolated from previous steps on agar medium supplemented with 5% cane sugar

D- Strain morphology isolated from previous steps under a 40. objective optical microscope

3.1.2. Evaluation of the effect of shaking time on yeast growth

The strain isolated from the previous steps was grown in a liquid YEPD medium, shaken at 180 Rpm, room temperature. From 3 hours after implantation, measure OD every 1 hour. The results showed that from the period of 1 to 8 hours of culture, the OD value was low, from the 17 hour period after culture, the OD value increased rapidly and remained stable up to 27 hours of culture. This result shows that from the 17-hour period after inoCulation, the yeast strain enters the logarithmic growth phase, the stage of yeast with high vigor, good division ability, which can be used as a secondary seed or cultured. inoCulation to increase biomass. However, due to limited time, growth assessments after 27 hours will continue to be conducted in the later stages of the study.

To initially determine the relationship between shaking culture time and biomass, 300 ml of shake culture medium was collected, yeast cells were collected, and bread dough production was tested. The results showed that after 2 hours of incubation, the volume of dough increased significantly. The powder is white, smooth, soft and elastic. However, further evaluation, and testing should be carried out in the next shaken culture periods.

Figure 2: Experimenting with bread production from yeast culture solution after 24 hours

3.2. Testing the possibility of bread production, using the obtained yeast strain

3.2.1. Testing the ability to increase powder volume of yeast strains obtained from fruit sources

The strain isolated from the previous steps was shaken in 500 ml of YEPD medium, used to test bread dough, and substitute for commercial yeast strains. The results showed that the strain was isolated from the previous steps was able to increase the volume of the dough.

Figure 3: test the ability generate CO2 make bread dough

During the incubation period of 1 - 2 hours, the volume of dough increases insignificantly, the dough is soft. At the 3-hour incubation time, the dough volume increased significantly. At the levels of 4 hours, 5 hours, 6 hours of incubation, the dough mass is soft and about doubled compared to the original. This result may be due to the low content of yeast cells in the early stage. The amount of CO_2 produced is not enough to change the volume of dough. Continuing to prolong the incubation time the yeast cells proliferate, which increases the volume of the dough mass.

To evaluate the potential application of the strain isolated from the previous steps in bread production, compare the experimental dough mass with the same mass of dough using a commercial baker yeast strain, after 7 - 8 hs, the dough bloCk containing strain isolated from previous steps and dough bloCk containing commercial yeast strain of equivalent size (Figure 4).

TM TH01 0

TM: Contains commercial yeast strains; TH01: Contains newly isolated yeast strains; O: Does not contain yeast

Figure 4: Assessment of CO2 generation making bread dough of strains isolated from previous steps

3.2.2. Evaluation of the effect of additive content in bread production

To evaluate the effect of bread additive content of dough, baking ability, flour bloCks was added with additives at the following concentrations: 0%; 1%; 5%; 15%; 20%. The results showed that, after 2 hours, additive at the concentration of 0%, the powder volume had a slight increase in volume, the surface was smooth and soft. At the concentration of 1% and 5%, the powder volume increased well, the dough mass was soft and elastic. However, the

surface of the dough was added 5% additive powder bloCk has many cracks and fractures (Figure 5B). The cause of this phenomenon is due to the large number of additives that reduce the elasticity of the dough. At the concentrations of 10% and 20% of the additive, the dough mass was stiff and poorly elastic (Figure 5A). Therefore, the additive content of 1% was selected for bread production.

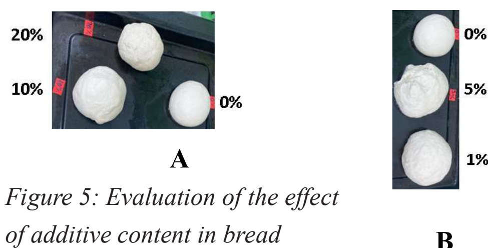

Figure 5: Evaluation of the effect of additive content in bread production

3.2.3. Evaluation of the effect of baking proCess of bread quality

To evaluate the influence of the baking proCess of the quality of bread, two types of equipment are used: SANAKY mini oven and industrial toaster oven. The results show that, at a baking temperature of 240 ^{0} C, time 20 minutes, industrial oven gives good results. The bread is yellow, bright, with a thin crust and a fatty aroma (Figure 6). With a mini oven, the bread is yellow, bright, has a fatty aroma, and the core is evenly cooked. However, the crust hardens quickly when the temperature is lowered. The cause may be due to the rapid dehydration at high temperature and the distance from the heat source of the mini oven too close.

Figure 6: Bread produced by strains isolated from previous steps, in an industrial oven

This result shows that, for each different type of equipment, there should be adjustments in the appropriate proCessing proCess. Experiments to optimize the proCess will be continued in the future.

4. Conclusion

Successfully isolated 01 yeast strain obtained from bananas for application for bread production. The possibility of bread production was tested for yeast strains obtained with good organoleptic quality.

REFERENCES

[1] Dung,N.L. (2010), *Textbook of Microbiology*, page 330.

[2] Phong,H.X., Loi,D.M., Thanh,N.N., Qui,L.P.D., Bui Hoang Dang Long,B.H.D., Pornthap Thanonkeo, Mamoru Yamada and Dung,N.T.P. (2017), Selection of heat-resistant yeasts and research on alcohol fermentation conditions pineapple wine, TU *Can Tho University Science Library* Episode 51, Part B (2017): 7 – 15.

[3] Oanh,V.T., Anh,P.T.H., Duong,V.T., Thuong,H.T. (2017), *Temperature control of fermenters and bread incubation* Journal of Science and Technology of Thai Nguyen University.

[4] Sadat Khattab, Ahmed Abdel-Hadi, Nageh Fathy Abo-Dahab, Omar Atta (2016), Isolation, Characterization, and Identification of Yeasts AssoCiated with Foods, *British Microbiology Research Journal,* 13.

[5] Theophilus Abonyi Mensah1and Sheila Matilda Ayorkor Tagoe (2019), Measurement of yeast growth using spectrophotometer, *Content uploaded by Theophilus Abonyi Mensah* .

[6] Tika B. Karki, ParashMani Timilsina, Archana Yadav, Gyanu Raj Pandey, Yogesh Joshi, Sahansila Bhujel, Rojina Adhikari, and Katyayanee Neupane1 (2017), Selection and Characterization of Potential Baker's Yeast from Indigenous Resources of Nepal, *Biotechnology Research International* , Volume 2017, Article ID 1925820.