International Journal on Advanced Science Engineering Information Technology

Vol.6 (2016) No. 3
ISSN: 2088-5334

Bread and Effervescent Beverage Productions with Local Microbes for the Local Revitalization

Manami Watanabe[#], Natsuki Uchida[*], Keiko Fujita[*], Tomoyuki Yoshino[*] and Toshifumi Sakaguchi[#*]

[#] *Department of Environmental Sciences, Prefectural University of Hiroshima, 562 Nanatsuka, Shoubara, Hiroshima, 727-0023, Japan*

** Department of Life Sciences, Prefectural University of Hiroshima, 562 Nanatsuka, Shoubara, Hiroshima, 727-0023, Japan*
E-mail: sakaguchi@pu-hiroshima.ac.jp

***Abstract*— Local microbes such as yeasts fungi and bacilli, were isolated and used to apply for the food processing (bread making) and the production of an effervescent beverage (fruits kvass) for promotion of the local revitalization. Our yeast isolates could contribute to make the breads that can sell in a local bakery shop. Furthermore, the same yeasts could be used for making the fruit kvass (a Russian effervescent beverage which was fermented by yeast) with local fruit products (lemon, dry grape, mandarin orange and apple that were produced in Hiroshima prefecture in Japan). Development of merchandise having the local brand, which was made with only products of Hiroshima prefecture was attempted. Results of these activities demonstrate that the search of local microbes can help to establish the regional brand product related to its local area. They also showed that the local microbes have the potential ability to lead the local revitalization and the local brand product.**

***Keywords*— Bread; Yeast; Effervescent Beverage (Fruit Kvass); Local Brand; Fermentation**

I. Introduction

Various microorganisms such as yeasts, fungi and bacilli have been used for the food processing and production of the effervescent or/and alcoholic beverages and the fermented foods manufacturing. They are great contributors in Asian cuisine. For example, baker's yeasts were widely used for making dairy breads [1]. From ancient times, yeasts also have been contributing to the production of various alcoholic beverages such as wine, beer, whisky, rum, vodka, sake and so on [2]–[4]. In others of the yeast, bacillus in the natto (fermented soy bean) production [5], koji-mold (aspergillus) in the saccharification and the amino acid production [6], and lactobacillus in the production of sour milk beverage, yogurt and fermented vegetables [7], [8] are indispensable microorganisms to produce our dairy fermented wholesome foods and beverages. Effective utilization of these microorganisms that isolated from the local area for development of the local fermented products can expect potential contribution for the promotion of local revitalization.

In this study, we report development of the new processed food and beverage products from local specialties by the fermentation of originally isolated microbes (the wild yeasts) from the local samples of north-Hiroshima area (Bihoku) for promotion of the local revitalization.

II. Experimental

A. Isolation Procedure of Microbes

For screening and isolation of wild yeasts, various samples were collected from the local area in north Hiroshima (Fig. 1). Mainly, agricultural products and biological samples from the fruits farms in the local area and the pilot farm in our university's campus (PUH Shoubara) were used as inocula of the isolation medium. For the isolation medium, modified YM medium (or plate) was used for the isolation of wild yeasts from the collected samples. To prevent the bacterial growth, lactate (120 µl per 1L YM medium) was added. The isolates were purified by the repetition of colony streaking to the fresh medium or plate, and then they were used for bread and fruit kuvass productions.

B. Fermentation and Growth Property

The yeast isolates were tested their fermentation ability with the gas trap chip kit (Eiken Kagaku; TX0210) based on Durham tube method for the screening of strains that had the high gas production ability. The growth curve of the selected yeasts for bread and beverage productions was investigated in YM medium. Growth was determined by measurement of optical density (OD) at 660 nm. Cell morphology and colony appearance of the selected isolates were observed.

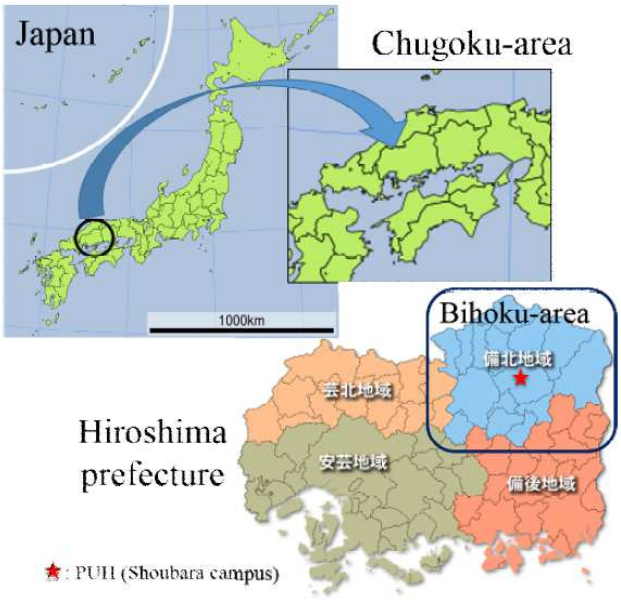


Fig. 1 Location of Bihoku (North-Hiroshima) in Japan

C. Bread Making and Beverage Production

The selected yeast cells were used for bread and fruits kuvass (a Russian effervescent beverage) productions. For evaluation of the produced bread, the unified recipe and materials was employed (Fig.2). Breads that were made by using our isolated yeasts, were measured their volumes (wide, length and height), and tasting and flavour analysis by gas chromatograph mass spectrometry (Shimazu; GCMS-QP5050, column; DB-WAX) were performed.

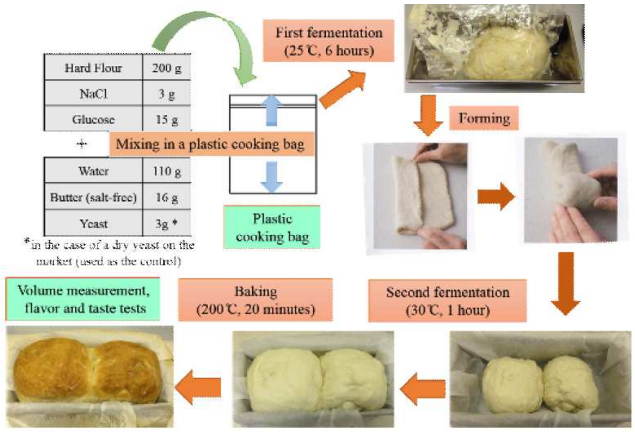
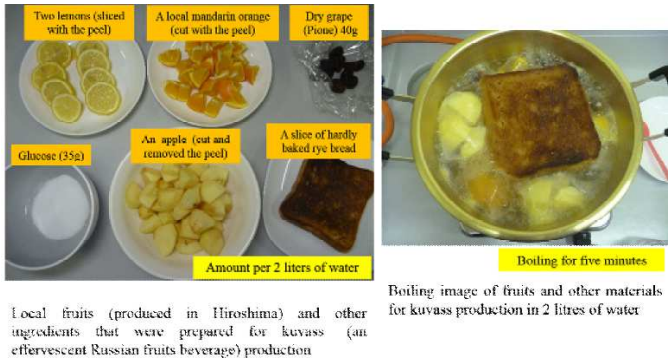


Fig. 2 Cooking recipe and materials for bread making

To make a fruit kuvass, local fruits (apple, lemon, dry grape and mandarin orange) that were produced in Bihoku-area and/or other areas in Hiroshima prefecture (Fig1) were prepared. Their fruits were cut into large pieces and then boiled with 2 litres of water for five minutes after addition of 35g glucose and a slice of hardly baked rye bread (Fig. 3). After cooling until room temperature our isolated yeast was added into the fruits extract. The fruits soup solution was incubated at 37℃ for 6 hours with shaking at 120 rpm for fermentation. The fermented solution was cooled at 4℃ and the then it was tasted and measured the sugar content and the acidity with a saccharinity refractometer (Atago; Pocket PAL-AC1) and an acidity test kit (Atago; Pocket PAL-J) respectively.



Local fruits (produced in Hiroshima) and other ingredients that were prepared for kuvass (an effervescent Russian fruits beverage) production

Boiling image of fruits and other materials for kuvass production in 2 litres of water

Fig. 3 Food materials and boiling process for kuvass production before fermentation

III. Results and Discussions

A. Isolation and Screening of Wild Yeasts

At least, 44 biological samples such as soils, wild plants and agricultural products were collected and over 50 crude microbial isolates were obtained by the rough screening of colony formation method. So far, at least twelve clones of pure yeast-like microbe were established by further repetition of the colony streaking. Due to further screening by the gas trap chip test, two strains of yeast (strains GLB-1 and PON-4) having gas production capability and suitable for fermentation and bakery yeasts were selectively separated from a variety of wild yeast isolates. Both strains were obtained from the biological samples (grapes; Delaware and Pione kinds) from the pilot farm in PUH Shoubara campus. Yeast like morphology and budding were observed by their phasecontrast photomicrograph observations (Fig. 4). Growth of each strain was followed by measuring optical density at 660 nm.

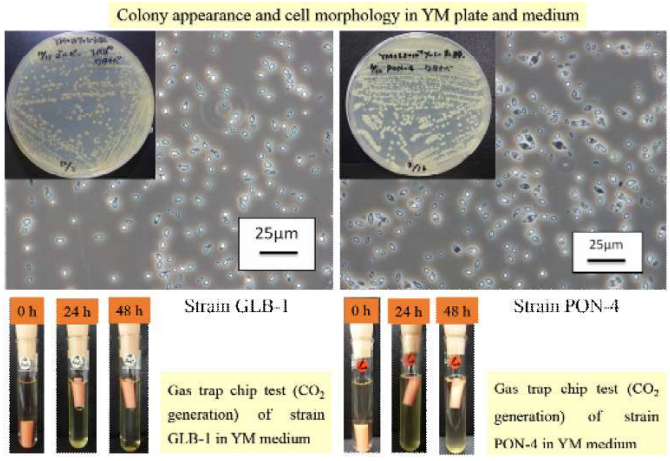


Fig. 4 Colony and cell morphologies and gas generation of strains GLB -1 and PON-4 that were isolated from grapes (Delaware and Pione kinds)

When 200 μl preculture of each strain (the cell density was approximately 1.2 -1.5 at OD660), which was grown in YM medium for about 24 hours, was employed as an inoculant of 250 ml YM medium in a 500 ml Erlenmeyer flask, each strain attained its late logarithmic or early stationary phase in 24 -28 hours in the case of GLB-1, and in 18 – 22 hours in the case of PON-4 respectively by the incubation at 25℃ with shaking at 120 rpm (Fig. 5). The cell

density (OD_{660}) reached to 1.0 – 1.4 (GLB-1) and 1.2 – 1.6 (PON-4) respectively (Fig. 5).

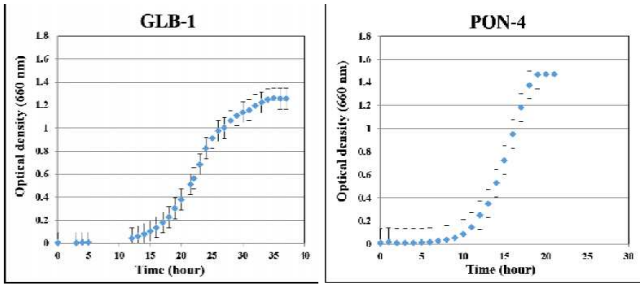


Fig. 5 Growth curve of strains GLB -1 and PON-4 that were grown in YM medium

In the comparison of both strains, PON-4 had a tendency that the time to attain its stationary phase become shorter than GLB-1. The maximum growth was higher than that of strain GLB-1. GLB-1 had a tendency that the lag phase become about five hours longer than that of PON-4. Both strains showed positive reaction within 24 hours to the gas generation test by the gas trap chip kit (Fig. 4). It was judged that these isolates had the fermentation capability enough for food processing. Each strain of cells corresponding to its late logarithmic or early stationary phase, which was cultured in YM medium, was used for bread making and fruits kuvass production. Now, we are progressing the phylogenetic analysis of both strains based on the sequence of the IPS region.

B. Evaluations of Produced Food and Beverage

Breads that made by using the wet cell paste of a wild yeast isolate (strains GLB-1 and PON-4) compared favourably with that of made by the dry yeast which is being marketed (Fig. 6).

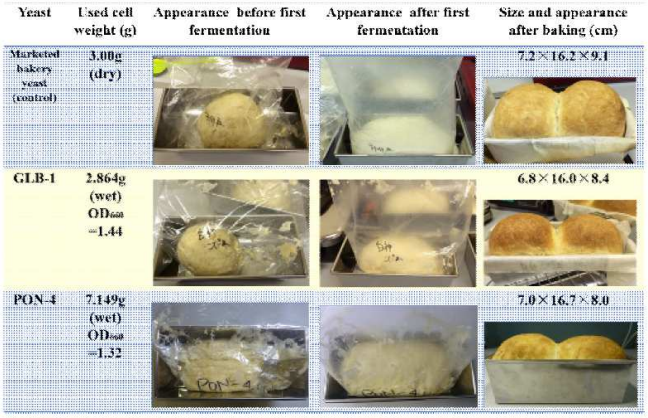


Fig. 6 Appearance and size of a bread made by using a wild yeast isolate (strains GLB-1 and PON-4)

The size after baking, flavor (impression test of real flavor) and taste were almost same with those of bread which made by the commercial bakery yeast. Additionally, the swelling degree of the plastic bag due to gas generation after the first fermentation could be used as an indicator for screening of the yeast suitable of making breads (Fig. 6). Flavor analysis based on the gas chromatography showed the presence of various flavor components in the odor form their breads. As referring to these results, we have determined the yeast isolate to use for making breads that can be supplied to a local bakery shop (mugimugi) in Bihoku area.

On the other hands, our isolated yeasts were also used for producing the fruits kuvass. By the addition of yeast, the effervescent beverage could be successfully made. The boiled fruits extract was changed as a smooth drink with sparking by the yeast's fermentation. Drastic changes on the acidity and the sugar content were not observed between before and after fermentation (Table 1). However, as shown in Table 1, the smooth taste might be caused by a slight decrease in the acidity and a slight increase in the saccharinity due to the fermentation.

TABLE I
CHANGE OF ACIDITY AND SUGAR CONTENT IN THE KUVASS MADE BY A WILD YEAST ISOLATE

Yeast (Addition: vol. or wt.)	Acidity (%: citrate equivalency) / Saccharinity (% Brix)		
	Cooked sample after boiling	Sample after incubation for 6 hours	Sample saved at 4℃ for 12 hours after incubation
PON-4 (200 µl culture @ OD_{660} 1.493)	n.d. / 4.9	n.d. / 4.9	n.d. / 5.7
PON-4 (200 µl culture @ OD_{660} 1.477)	n.d. / 4.9	n.d. / 5.1	n.d. / 6.0
GLB-1 (200 µl culture @ OD_{660} 1.077)	n.d. / 4.9	n.d. / 5.3	n.d. / 5.8
PON-4 (0.09 g dry cells @ OD_{660} 0.991)	0.23 / 4.4	0.28 / 5.2	0.27 / 5.2
PON-4 (0.06 g dry cells @ OD_{660} 0.991)	0.23 / 4.4	0.27 / 5.2	0.27 / 5.2
No addition (control)	0.23 / 4.4	0.30 / 4.8	0.30 / 4.6

n. d. : not determined

Further investigations such as measurements of the alcohol concentration and the antioxidant activity are now underway for the establishment of a regional brand in Bihoku area.

C. Possibility of Local Branding

To explore the possibility of local branding of breads made by a wild yeast isolate, manufacture of the commercial breads with our isolated wild yeasts (strains GLB-1 and PON-4) was carried out in a local bread shop (in mugimugi's antenna shop; green mugimugi in Torreta Miyoshi, Miyoshi city, Bihoku area of Hiroshima, shown in Fig. 7). Our yeast isolates could contribute to make several kinds of bread which can be supplied to the local bakery shop (Fig. 7).

Fig. 7 Breads that made by all locally made (and/or found) products in Bihoku area and our isolated yeast (strain GLB -1 or PON-4) (shown in the left), and Toretta Miyoshi (mugimugi 's antenna shop; green mugimugi) (shown in the light)

The local bakery shop mugimugi has a policy that the all bread should be made by the locally made wheats (in

Miraska of Bihoku). Our attempt could lead to the birth of merchandise having the local brand, that made by all locally made (and/or found) products. This result also showed the possibilities that our trail can be an initiator for reevaluation and value formation of the local farm products, and that such action furthermore promotes local production for local consumption and understating of food mileage [9],[10].

IV. Conclusions

In this reports, through the case of yeast, we have been describing about the isolation and the utilization of local microbes for the bread making and the production of an effervescent beverage (fruits kvass). These activities could be lead to promote the local revitalization and to produce merchandise having the local brand, that made by all locally made or found products. They also showed that the local microbes have a potential ability to lead the revitalization of the depopulated areas in local sites of Hiroshima, Bihoku. Our attempt to give rise to the potential ability of local-origin microbes, could lead to the reevaluation of local agricultural products. Further food processing and production with the local microbes except yeasts is the future subject expected.

Acknowledgment

This work was conducted by the financial support of "President Project in the essential research of PUH Shoubara campus". M.W. and T.S. also gratefully thank to a bakery shop and studio (boulangerie) in Bihoku, "mugimugi" owner & chef, and the bread master, Mr. Satoshi Nojima and the chief director, Ms. Chinatsu Nojima for providing techniques on bread making and opportunities of the bread sale at the shop.

References

[1] L. S. Young and S. P. Stanley, Technology of Breadmaking (ISBN:0-387-38563-0), Berlin, Germany: Springer-Verlag, Cap. 3.7; Barker's yeast, pp. 76-88, 2007.

[2] M. Battock and S. A. Ali, Fermented Fruits and Vegetables: A Global Perspectives (ISBN-13: 978-8170352488), Delhi, India: Daya Publishing House, Cap 3; Yeast fermentation, and Cap. 4; Products of yeast fermentation, 2001.

[3] P. E. McGoverna, A. Mirzoian, and G. R. Halla, "Ancient Egyptian herbal wines", PNAS, vol. 106, 7361–7366, 2009.

[4] P. E. McGovern, J. Zhang, J. Tang, Z. Zhang, G. R. Hall, R. A. Moreau , A. Nunez, E. D. Butrym, M. P. Richards, C.-S. Wang, G. Cheng, Z. Zhao, and C. Wang, "Fermented beverages of pre- and proto-historic China", PNAS, vol. 101, 17593–17598, 2004.

[5] W. Shurtleff and A. Aoyagi, History of Natto and its Relatives (ISBN: 978-1-928914-42-6), Lafayette, USA: Soyinfo Center, pp. 23-593, 2012.

[6] K. Abe and K. Gomi, "The Aspergilli: Genomics, Medical Aspects, Biotechnology, and Research Methods" (ISBN-13:978-0-8493-9080-7), G. H. Goldman and S. A. Osmani, Ed., Florida, USA: CRC Press, Taylor & Francis Group, Cap. 25; Food Products fermented by Aspergillus oryzae, pp. 429-440, 2008.

[7] K. H. Steinkraus, "Lactic acid fermentation in the production of foods from vegetables, cereals and legumes", Antonie van Leeuwenhoek, vol. 49, 337-348, 1983.

[8] Y. Widyastuti , Rohmatussolihat and A. Febrisiantosa , "The Role of lactic acid bacteria in milk fermentation", Food and Nutrition Sciences, vol. 5, 435-442, 2014.

[9] T. Lang, 'locale / global (food miles)', Slow Food (Bra, Cuneo Italy), 19, May, p.94-97, 2006.

[10] C. Barclay, "Food Miles", in a PDF, (http://researchbriefings. files.parliament.uk/documents/SN04984/SN04 984.pdf), 2012.