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Review

Microbiota Associated with the Starter Cultures and Brewing Process of Traditional *Hong Qu* Glutinous Rice Wine

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Abstract *Hong Qu* glutinous rice wine (produced mainly in Fujian province, China) is a traditional alcoholic beverage, which is prepared by fermenting cooked rice using a starter containing *Monascus purpureus*. In this review, the microbial diversity of fermentation starters from Fujian province, including fungi, bacteria, and yeast, is analyzed in comparison with those of "*nuruk*" (a traditional starter for making alcoholic beverages in Korea). The bacterial organization of *Hong Qu* starters was vastly variable in species composition and dominated by *Bacillus* sp. Lactic acid bacteria were also found in some starters. In case of fungi, *Monascus* sp. was dominant, whereas non-*Saccharomyces* yeast such as *Saccharomycopsis fibuligera* was detected. The microorganisms found in the *nuruk* starter are, in general, not significantly diverse compared with those found in the *Hong Qu* starter, with the exception of *Monascus* sp.; however, *Hong Qu* and *nuruk* both contain their own unique microbiota, which are quite diverse from each other.

Keywords: *Hong Qu* starter, *Hong Qu* rice wine, *nuruk*, microbiota, *Monascus*

Introduction

Jiuqu is known as starter cake but more generally recognized as *Qu* (English pronunciation Chew), which is a type of East Asian fermentation starter that is grown on a solid substrate and utilized in the production of traditional alcoholic beverages in China. In many Asian countries, *Qu* is known by other names: *koji* in Japan, *nuruk* in Korea, *murcha* in India, *banh men* in Vietnam, *loog pang* in Thailand, *ragi* in Indonesia and Malaysia, and *bubod* in the Philippines (1). The process of preparing *Qu* for utilizing in fermentation is considered to have originated in China around 3,000-4,000 years ago and has since been introduced to other nations in Asia (2-4). The preparation of *Qu* includes a procedure of growing microbes on starchy materials and is a completely separate process that precedes the fermentation of grains into alcoholic beverages. Traditional preparations are often empirical, take place in homes and small-scale manufacturing facilities, and vary from region to region. Traditionally, *Hong Qu* is used in the fermentation of polished round grain rice by inoculating *Monascus* sp. strains under nonsterile conditions. In contrast, white *Qu* is manufactured from starchy substrates originating from conventional agricultural fields; in addition, a Chinese native medicinal ingredient may be added in a prescribed quantity (4).

On the other hand, "*nuruk*" is a traditional microbial starter material for brewing alcoholic beverages in Korea, such as "*takju*" ("*Makgeolli*"), "*cheongju*," and "*yakju*." Many kinds of *nuruks* exist, and most are made from the uncooked dough of coarsely ground grains and water. They are combinations of mixed cultures including filamentous fungi, yeast, and bacteria grown on a variety of cereal grains. Barley, millet, soybean, and nonwaxy rice are the most popular materials used in Korea, whereas glutinous rice is the most popular cereal used for preparing the starters in China (5).

Chinese yellow rice wine can be classified into three main groups: (i) *Hong Qu* glutinous rice wine (produced mainly in Fujian province), (ii) wheat/yeast rice wine (represented by *Shaoxing* rice wine produced in Zhejiang province), and (iii) millet yellow wine (symbolized by *Jimo* old in Shandong province). Red yeast rice (called as "*Hong Qu*" or "*angkak*" in Chinese) is produced by fermenting *Monascus purpureus* with rice and liquor brewed from glutinous rice with the inoculation of the red yeast rice starter (*Hong Qu*). This has been referred to as *Hong Qu* glutinous rice wine and is a famous traditional fermented product in the southeast of China and Southeast Asia (2). The use of red yeast rice in China was first recorded during the Tang Dynasty in 800 A.D. A detailed manufacturing process is described in the ancient Chinese pharmacopeia, Ben Cao Gang Mu-Dan by Shi Bu Yi, published during the Ming Dynasty (1,368-1,644) and is also found in Dong Eui Bo Gam by Huh Jun (1,539-1,615) from the Lee Dynasty in Korea. In

this book, a mild red yeast rice was recommended as a mild aid for gastric problems, blood circulation, and the promotion of gastrointestinal health (2-4).

Chinese yellow rice wine is brewed from rice with the addition of fermentation starters, which include a large diversity of enzymes and microorganisms, including filamentous fungi, yeasts, and bacteria (6,7). Similarly, the starter cultures, both of *Hong Qu* and *nuruk*, where numerous microorganisms, such as fungi, bacteria, and yeasts can be grown, support amylolytic and proteolytic enzyme sources, which are necessary for the fermentation of rice wine. However, similar to many other traditional fermented foods, it is manufactured or processed with uncontrolled fermentation under nonsterile conditions; this process is based on traditional practical knowledge, and remains a traditional skill at homes and small-scale industries (5,8,9).

Many attempts have been made to quantify and determine the microbial diversity of rice wine starters and rice wine using classical, physiological, and morphological observations. In recent studies, however, combinations of molecular analyses and classical methods were used to more effectively identify the microbial diversity. Thus, the microbial diversity correlated with traditional fermentation starters for *Hong Qu* has been investigated by performing morphological, physiological, and phylogenetical analyses; polymerase chain reaction (PCR); and PCR-mediated denaturing gradient gel electrophoresis (DGGE) (10-14).

Furthermore, the *Hong Qu* glutinous rice wine has a bright-red color, fine sweet flavor, and healthcare functionality, primarily because of the addition of the red yeast rice starter. Both *Hong Qu* and red yeast rice can considerably decrease total cholesterol, low-density lipoprotein (LDL) cholesterol, and total triacylglycerol concentrations while exhibiting antioxidant activities (15-19). Thus, *Hong Qu* or red yeast rice has been historically used as a food colorant and blood circulation treatment agent (20-27).

The aim of this review is to briefly summarize the available information on *Hong Qu* glutinous rice wine, traditionally produced in Fujian Province, China, by focusing on its microbial diversity and dynamics. In addition, the diversity of microbiota present in fermentation starters appears to be an essential factor in determining the specific type of rice wines. The dominant species is highly dependent on the substrate, climate, and processing techniques. Thus, the comparison of these factors in *Qu* may provide a better understanding of the present status of rice wine production in East Asian countries.

The traditional brewing process of *Hong Qu* glutinous rice wine

***The production process of fermentation starters*:** There is little information available on the production process of fermentation starters because the techniques are often practiced as a hereditary trade that is secretly passed down from parents to children (8). The major principles in manufacturing fermentation starters include: (i) preparation of cereal grains (mostly rice), (ii) inoculation with previously grown microbiota, (iii) incubation for a specific period to stimulate growth and metabolism of microorganisms, and (iv) drying for preservation (Fig. 1). Cereals used for the production of the *Hong Qu* fermentation starters are different from others. Traditionally, *Hong Qu* is fermented from polished round grain rice, whereas others, like white *Qu*, are processed from starch substrates originating from common agricultural areas; sometimes various Chinese native herbs are also added. Glutinous rice is then soaked in water for 30 min, pounded, and mixed with an old starter powder (1-2%). It is then moved to an incubation room (35-40°C) and incubated for 4-5 days, and then, the product is dried under sunlight (10,11,14). Mixing new starters with old ones seems to be an important step for inoculation of *Monascus* sp., although aging is the process for natural inoculation for various microorganisms (11).

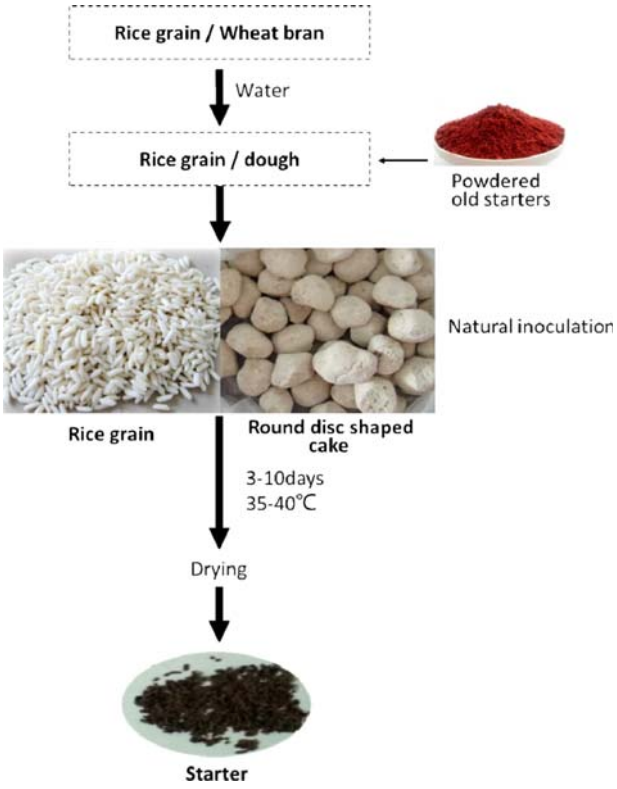

Fig. 1. The traditional production process of *Hong Qu* fermentation starters

***The brewing process*:** The traditional process of brewing varies across regions and significantly diverges from the ideal process now applied to prepare the more modern industrialized rice wine. The major principles applied in manufacturing *Hong Qu* are described in Fig. 2 (13). Glutinous rice was soaked and steamed and then cooled to room temperature. To initiate the brewing process, steamed rice was mixed with the fermentation starters in a container in which *Hong Qu* was soaked in water for 7-8 h prior to mixing. After fermenting at 15-20°C for at least 30 days, the fermented mash was filtered. After collecting, clarifying, and sterilizing the rick wine, it was retained for aging. In the 21st century, complete aseptic laboratory

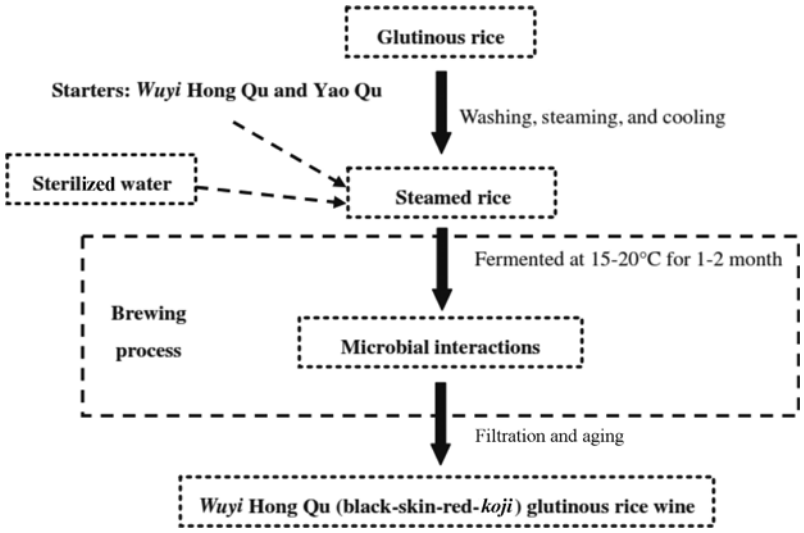

Fig. 2. Flow chart for the traditional brewing of *Hong Qu* glutinous rice wine (13)

techniques and conditions were introduced to monoculture-specific favorable strains of bacteria, yeast, and mold grown on substrates (4).

Microorganisms associated with traditional fermentation starters

***Filamentous fungi*:** Many studies have investigated the microbial diversity of various traditional fermentation products, including the rice wine fermentation starters (28-30). However, these studies determined the microbial community of wine starters by employing a culture-dependent method. Because of this, some significant microbial species whose cultural growth prerequisites were undetermined and lost, thus inhibited from establishing the whole picture of the microbial community present in wine starters. Molecular culture-independent approaches based on 16S and 18S rRNA gene analyses have proven to be powerful tools in acquiring a more complete inventory of the microbial diversity in food samples compared with the conventional culture-dependent methods (5,11,30-45). Numerous studies have been performed to more accurately describe the microbial diversity of rice wine and fermentation starters (6,7,46-49). It has recently been reported that *Monilia*, *Mucor*, *Rhizopus*, *Absidia (Lichtheimia)*, *Monascus*, *Aspergillus*, and *Penicillium* are the most common filamentous fungi in Chinese yellow rice wine starters (6). Sixteen kinds of filamentous fungi were separated from wheat *Qu*, a starter of *Shaoxing* rice wine, and identified by internal transcribed spacer (ITS) sequencing technology (39).

Lv *et al.* (14) have characterized 43 filamentous fungi, which were separated from 10 fermentation starters, using macroscopic and microscopic characteristic methods and were then classified into 16 different species on the basis of morphological determination and ITS sequences analysis. Among them, the genus *Aspergillus* had the most popular number (14 isolates) of isolates, followed by *Rhizopus* (11 isolates), *Monascus* (5 isolates), and *Penicillium* (4 isolates). The species *Rhizopus oryzae*, *A. niger*, *A. flavus*, and *M. purpureus* were commonly found in wine starter samples, among which *R. oryzae* was the most prevalent species.

As depicted in Table 1, *Hong Qu* and white *Qu* differed in the main filamentous fungi. Three fungi species (*A. niger*, *M. purpureus*, and *A. flavus*) were widely found in Hong Qu, of which, *A. niger* and *M. purpureus* were only detected in *Hong Qu*. The microbial diversity of white *Qu* was more complex than that of *Hong Qu*, suggesting that *M. purpureus* in *Hong Qu* would have obvious effects on microbial diversity (50,51). Some fungal species were only detected in white *Qu*, including *Mucor circinelloides*, *Paecilomyces* sp., *Ascomycota* sp., and *R. arrhizus.* This may be because of the antimicrobial substances produced by *Monascus* sp., thus, creating conditions unfavorable for the growth of other microorganisms.

On the other hand, Yu *et al.* (52) comprehensively reviewed the literature on microbial diversity of traditional Korean *nuruk*, which has been published since 1945. Traditional Korean *nuruk* is composed of unboiled raw barley and various other grains; therefore, a variety of microorganisms, such as fungi, yeasts, and bacteria, grow in *nuruk* (53-55). The total number of fungal species identified was up to 38 species, including 14 species of *Aspergillus*, nine species of *Penicillium*, five species of *Candida*, four species of *Hansenula*, one species of *Pichia*, and one species of *Schizosaccharomyces*. Similarly, other preceding studies have shown that Eurotiales and Mucorales members, including the genera *Aspergillus*, *Lichtheimia* (formerly *Absidia*), *Rhizopus*, *Rhizomucor,* and *Mucor,* are the most frequently identified fungi from *nuruk* (Table 1, 56). Recently, the integrated morphological, physiological, and phylogenetic analyses on the fungal isolates from *nuruk* samples have been performed (5): 174 fungal isolates were isolated from 39 *nuruk* samples. Mucorales was the most popular mycobiota in *nuruk*, followed by yeast and *Aspergillus*. Six genera, i.e., *Aspergillus*, *Lichtheimia*, *Mucor, Rhizopus*, *Rhizomucor,* and *Syncephalastrum*, and 17 fungal species were identified. Two genera, *Aspergillus* and *Lichtheimia*, comprised

Table 1. Fungal species composition of various rice wine starters (5,14). Nonshaded, fungus not found in rice wine starter; Shaded, fungus found in rice wine starter

Wine starter	White *Qu*					Red *Qu*					*Nuruk*				
Species	I*	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mucor circinelloides	■										■	■	■		
Uncultured eukaryote clone			■	■											
Lichtheimia ramosa							■				■	■	■	■	■
Aspergillus niger						■	■	■	■	■	■	■	■		
Penicillium chrysogenum				■			■	■	■						
Monascus sp.						■	■	■	■						
Rhizopus oryzae	■	■	■		■		■	■	■	■	■	■	■		
Rhizopus microsporus		■				■					■				
Paecilomyces sp.		■		■											
Aspergillus tubingensis				■							■				
Mucor indicus					■										
Aspergillus oryzae					■						■	■	■	■	■
Aspergillus flavus					■	■	■	■	■	■	■	■	■		
Ascomycota sp.		■		■											
Rhizopus arrhizus				■											
Emericella nidulans					■										
Lichtheimia corymbifera											■	■	■	■	
Rhizomucor pusillus											■	■	■	■	

*Roman numbers indicate different sources of samples.

almost 84% of the filamentous fungal isolates from the *nuruk* samples, and *Aspergillus oryzae* and *Lichtheimia ramosa* were the most frequently occurring species.

Recently, our group collected 19 *nuruks* from various regions in Korea and isolated 100 fungal strains utilizing a culture-based method (data not shown). The most popular fungal strain was the *Aspergillus* sp. followed by *Lichtheimia* sp., similar to the report by Yang *et al*. (5). *A. oryzae* (17/100) was the prevalent fungal species followed by *L. corymbifera* (12/100) and *A. flavus* (8/100). Other fungal strains found were *Mucor* sp., *Rhizomucor* sp., *Emericella* sp., *Eurotium* sp., *Clavispora* sp., *Irpex* sp., *Penicillium* sp., *Galactomyces* sp., and *Geotrichum* sp.

Nuruk contains unique mycobiota that are not yet identified in *Hong Qu* starter cultures. In Table 1, the results were summarized by re-editing at the level of the previous table of Lv *et al.* (5,14). Most members of fungi found in *nuruk* are shared with those of white *Qu* and red *Qu*; however, some isolates of the *Lichtheimia* sp. and *Rhizomucor* sp. are present only in *nuruk*, which exhibited different results from those of white *Qu* and red *Qu*.

***Yeast diversity*:** The yeast flora existing in the *Hong Qu* fermentation starter has been investigated using both culture-dependent and culture-independent molecular biological methods (10). By employing a combination of polymerase chain reaction-restriction fragment length polymerization (PCR-RFLP) analysis of the ITS1-5.8S-ITS2 and sequencing of the D1/D2 domain of the 26S rRNA gene, molecular identification of a total of 500 yeast isolates from 10 different types of representative wine fermentation starters was completed. The sequencing analysis of 13 different ITS/RELP profiles resulted in 12 different species, belonging to eight different genera: *Pichia, Saccharomyces, Candida*, *Cryptococcus, Rhodotorula, Sporobolomyces, Rhodosporidium*, and *Saccharomycopsis* (Table 2). *Saccharomycopsis fibuligera* was the most commonly isolated species, followed by *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* was also detected in low frequency at the early stage of traditional brewing. This is in agreement and compatible with the low numbers of this species, which are present in the wine fermentation starters (57). Non-*Saccharomyces* yeasts, such as *Saccharomycopsis fibuligera* and *P. guilliermondii*, increased significantly during the early period of brewing, whereas *Saccharomyces cerevisiae* increased and dominated toward the middle of the fermentation process. On the other hand, Ha *et al*. (58) isolated and characterized the starch-utilizing yeasts from *nuruk*, which appeared to be somewhat differentiated from other glucose-utilizing yeasts.

Recently, we have isolated 47 yeast strains based on a culture-based method from four different *nuruk* samples in Korea (Table 2, data not shown). The most popular yeast strain was the *Saccharomycopsis* sp. followed by *Wickerhamomyces* sp. Among those, *Saccharomycopsis fibuligera* (22/47) was the most prevalent fungal species followed by *Wickerhamomyces anomalus* (13/47). The yeast species most frequently reported for traditional Asian fermentation starters was *Saccharomycopsis fibuligera* (59). Interestingly, only one *Saccharomyces cerevisiae*, known to be the most popular alcohol fermentation yeast, was isolated. It is quite interesting that *Saccharomycopsis fibuligera* was the most predominant yeast strain in both *nuruk* and *Hong Qu*. These observations indicated that wild yeast strains other than *Saccharomyces cerevisiae*, are the actual alcohol fermentation yeasts to make oriental alcoholic beverages. Other yeast strains found were *Aureobasidium* sp., *Cryptococcus* sp.,

Table 2. List of yeasts isolated from *nuruk* (a) and *Hong Qu* glutinous rice wine starters (b) (10)

Yeast species[a]	Strain numbers identified	Yeast species[b]	Strain numbers identified
Aureobasidium pullulans	1	*Candida glabrata*	1
Cryptococcus albidus	2	*Cryptococcus heveanensis*	1
Cryptococcus liquefaciens	1	*Cryptococcus albidus*	1
Cryptococcus sp.	2	*Pichia fabianii*	1
Pichia jadinii	1	*Pichia guilliermondii*	2
Pichia anomala	1	*Rhodosporidium toruloides*	2
Rhodotorula mucilaginosa	1	*Rhodotorula mucilaginosa*	2
Saccharomyces cerevisiae	1	*Saccharomyces cerevisiae*	5
Saccharomycopsis fibuligera	22	*Saccharomycopsis fibuligera*	8
Sporobolomyces sp.	1	*Saccharomycopsis malanga*	3
Wickerhamomyces anomalus	13	*Sporobolomyces nylandii*	2
Wickerhamomyces sp.	1	*Wickerhamomyces anomalus*	2
Total	47	Total	30

Table 3. Bacterial strains isolated from traditional fermentation starters for *Hong Qu* glutinous rice wine (11)

Bacterial strains	Accession No.
Bacillus amyloliquefaciens	CP002927
Bacillus aryabhattai	JF951729
Bacillus aryabhattai	HQ857752
Bacillus ginsengihumi	HQ219845
Bacillus megaterium	HE578782
Bacillus megaterium	JN106424
Bacillus methylotrophicus	HQ844510
Bacillus methylotrophicus	JF460760
Bacillus subtilis	JN382471
Bacillus subtilis	JF719789
Janthinobacterium lividum	JF970593
Pediococcus acidilactici	HQ603181
Pediococcus pentosaceus	JN039354
Pediococcus pentosaceus	HQ589249
Uncultured bacterium clone	JH719426
Uncultured bacterium clone	JF329433
Weissella paramesenteroides	HQ721270

Pichia sp., *Rhodotorula* sp., and *Sporobolomyces* sp.

***Bacterial diversity of traditional starters for Hong Qu glutinous rice wine*:** The bacterial DGGE profile indicated that the bacterial diversity of starters was highly variable in species composition and was predominated by *Bacillus* sp., including *B. ginsengihumi*, *B. megaterium*, *B. aryabhattai*, *B. subtilis*, *B. methylotrophicus*, and *B. amyloliquefaciens*. Lactic acid bacteria (LAB), including *Weissella paramesenteroides*, *Pediococcus pentosaceus*, and *P. acidilactici*, were also detected in some fermentation starters (Table 3). LAB has been discovered in various wine fermentation starters, such as *nuruk*, *ragi*, and *tape* and traditional Vietnamese rice wine starters (34,54). It is noteworthy that LAB is not affected by the bacteriostatic effect of *Monascus fermentate* (15), although gram-positive bacteria are generally more inhibited than gram-negative bacteria.

***Enzyme activity of fungi from starters*:** The fermentation starters are used as a source of hydrolytic enzymes, such as starch hydrolases and proteases. In particular, glucoamylase and α-amylase are two important enzymes considered necessary for starch hydrolysis. However, the properties and characteristics of these enzymes are poorly understood, and the detailed data for the progress of enzyme reactions during the fermentation process have not been effectively studied. Recently, the ability to produce α-amylase, glucoamylase, and protease has been examined on the species level while using the isolated filamentous fungi in the evaluation of different starters (14). *A. flavus*, *M. purpureus*, and *R. oryzae* are known to be superior glucoamylase producers. *A. flavus*, *R. oryzae*, and *A. oryzae* exhibit higher activity of α-amylase. *A. flavus* and *A. oryzae* have higher protease activity. Results are shown in Table 4 (14).

With respect to α-amylase activity, the species *A. flavus* is the best producer followed by *A. oryzae* and *R. oryzae*. *Monascus* sp. had high glucoamylase activity in contrast to low α-amylase activity. *Ascomycota* sp., *M. circinelloides*, and *M. indicus* did not produce α-amylase, although they depicted slight glucoamylase activity. With respect to protease activities, *A. oryzae* was the best producer followed by *E. nidulans* and *A. flavus*. There were no significant differences among the species *R. oryzae*, *R. arrhizus*, and *Monascus* sp. for protease production, all of which showed a moderate level of protease activities.

The alteration of enzyme activities during fermentation has been investigated in *nuruk* (56,60). Acid and neutral protease activities were considerably increased, but alkaline protease activity was not detected. Thus, α-amylase activity was progressively increased and reached the maximum level of approximately 2,833,000 U/g after a 15-day fermentation process, whereas glucoamylase activity was approximately 497.9 U/g. The degradation of allergenicity in *nuruk* fermentation was supposed to decrease allergic proteins in wheat. Similarly, Dung *et al*. reported that *R. oryzae* in mold isolates from a Vietnamese rice wine starter exhibited an excellent glucoamylase ability (9). The species *A. flavus* showed similar glucoamylase activity levels to that of *R. oryzae*. There were no significant differences among the species *A. flavus*, *M. purpureus*, *Paecilomyces*, *R. oryzae*, and *R. arrhizus* for glucoamylase production, all of which exhibited

Table 4. Mean values and standard deviations of glucoamylase, α-amylase, and protease activities (U/g) produced by different fungal species obtained from different wine starters (14)

Species (number of strains)	Glucoamylase activity	α-Amylase activity	Protease activity
Aspergillus flavus (6)	3,314.92±1,657.03$^{ab1)}$	9,155.17±4,866.04^{a}	227.36±91.74^{b}
Aspergillus oryzae (1)	1,982.37±149.55^{bcdef}	2,461.55±213.63^{b}	307.41±4.22^{a}
Aspergillus tubingensis (1)	1,544.01±38.77^{cdefg}	443.36±9.62^{b}	1.68±2.37^{e}
Ascomycota sp. (2)	766.34±35.33^{fg}	ND$^{2)}$	26.51±2.68^{de}
Aspergillus niger (5)	1,425.07±169.00^{cdefg}	386.71±45.70^{b}	0.30±0.83^{e}
Emericella nidulans (1)	939.88±18.00^{fg}	598.46±27.76^{b}	280.73±0.2^{ab}
Lichtheimia ramosa (1)	1,564.58±23.54^{cdefg}	839.34±49.73^{b}	35.63±2.90^{de}
Mucor circinelloides (1)	267.22±47.08^{g}	ND	ND
Monascus sp. (5)	2,437.24±379.17^{abcde}	322.10±36.30^{b}	82.55±11.78^{cde}
Mucor indicus (1)	379.82±15.23^{g}	ND	ND
Penicillium chrysogenum (4)	939.78±268.77^{fg}	330.08±209.32^{b}	121.01±85.62^{c}
Paecilomyces sp. (2)	2,613.72±206.48^{abc}	1,718.91±484.43^{b}	39.08±18.00^{de}
Rhizopus microsporus (2)	1,117.11±226.76^{defg}	247.68±38.54^{b}	44.67±5.30^{cde}
Rhizopus oryzae (8)	3,609.30±830.15^{a}	2,592.75±727.56^{b}	98.33±10.81^{cd}
Rhizopus arrhizus (1)	2,504.55±18.00^{abcd}	1,951.69±163.03^{b}	89.72±2.37^{cd}
Uncultured eukaryote clone (2)	1,058.13±29.87^{efg}	273.80±12.30^{b}	37.23±1.91^{de}

[1)]Mean values for three independent experiments. Values are presented as means±SD (n=3); values within the same column in each characteristic with different letters are significantly different by Duncan's multiple range test (p<0.05).
[2)]ND, Not detected because the enzyme produced by fungi isolates was too low.

high glucoamylase activities. Yoshizaki *et al*. (61) examined the effect of different culture conditions (solid culture) on enzyme production and characterized the glucoamylase and α-amylase from *Monascus anka*. The influence of temperature revealed that incubation at 37°C stimulated maximal cell growth, whereas incubation at 25 and 40°C resulted in increased α-amylase and glucoamylase production, respectively. *Monascus anka* shows a glucoamylase activity of 409.6 U/mL under optimal conditions in a submerged culture (31).

Microbial community dynamics during fermentation

***Bacterial community dynamics during fermentation*:** The bacterial community dynamics was thoroughly investigated during the traditional fermentation of *Wuyi Hong Qu* glutinous rice wine using PCR-DGGE and 16S rRNA gene clone library analysis (13). The principal bacterial species in the traditional wine fermentation starters are *Pediococcus pentosaceus*, *P. acidilactici*, and *Bacillus* sp. (including *B. aryabhattai*, *B. megaterium*, and *B. amyloliquefaciens*). The bacterial community dynamic revealed the presence of *Bacillus* sp. and LAB (including *Lactobacillus plantarum*, *L. brevis*, *P. acidilactici*, and *P. pentosaceus*) during the fermentation process; however, they changed in different brewing stages. Some other bacterial species, such as *Bacillus* sp., *P. acidilactici*, *L. brevis*, and *P. pentosaceous*, were detected at an early stage but decreased as the fermentation progressed. However, the *L. plantarum* group was constantly detected throughout the fermentation process. The result reflected a significant agreement with a previous study, in that LAB is not affected by the bacteriostatic effect of *Monascus fermentate* (15).

***Dynamics of fungal and yeast communities during fermentation*:** Lv *et al*. (12) characterized the fungal community dynamics during the traditional brewing of *Wuyi Hong Qu* glutinous rice wine. The relative proportions of some various fungal species, including *R. oryzae*, *Pichiaguillier mondii*, and *Saccharomycopsis fibuligera*, were detected in the early brewing stage; however, they significantly decreased as the fermentation progressed, whereas *Saccharomyces cerevisiae* became the dominant species during the latter fermentation stage (Fig. 3). *A. oryzae* decreased continuously and maintained a relatively small population throughout the entire brewing process (12). In contrast, the concentration of *M. purpureus* gradually increased during the early period of the brewing process but decreased slowly between days 10 and 46, as revealed in Fig. 3.

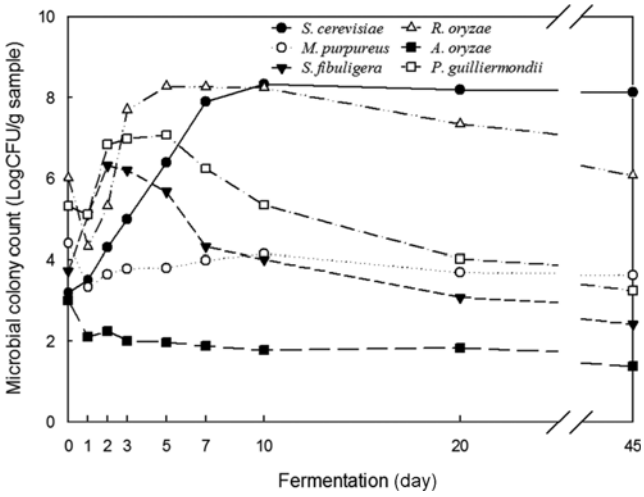

Fig. 3. Profile of the quantitative changes of some dominant fungi species during *Wuyi Hong Qu* glutinous rice wine fermentation process (12).

Saccharomyces cerevisiae was also detected at a low frequency at early stage of traditional brewing, thus confirming the low numbers

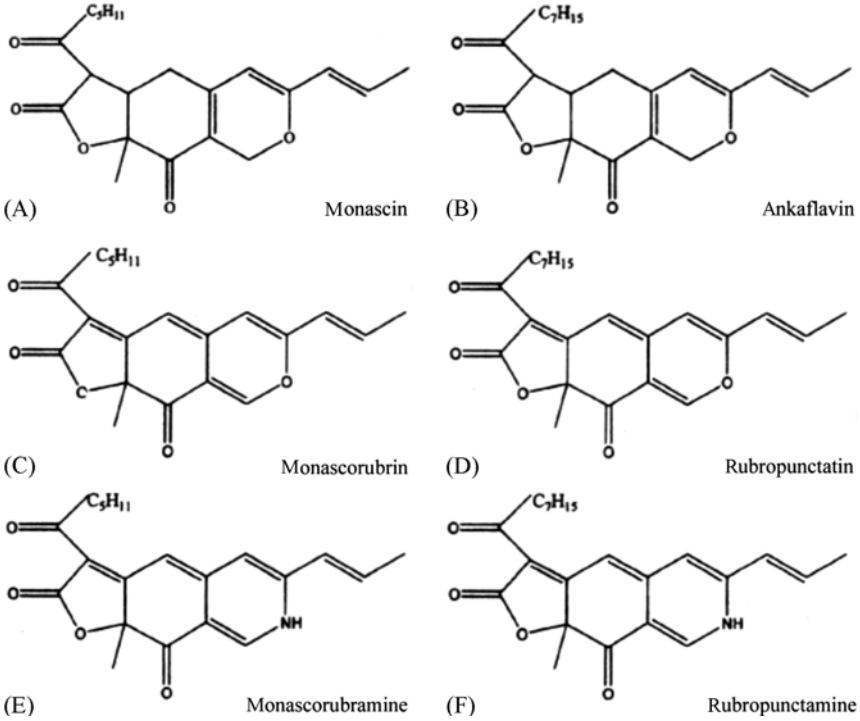

Fig. 4. Chemical structures of *Monascus* pigments (20). (A) and (B), yellow pigments; (C) and (D), orange pigments; and (E) and (F), red pigments.

of this species in wine fermentation starters. However, *Saccharomyces cerevisiae* increased during the fermentation processes because of its ethanol tolerance. Non-*Saccharomyces* yeasts, such as *Saccharomycopsis fibuligera* and *P. guilliermondii*, increased significantly during the early period of brewing, whereas *Saccharomyces cerevisiae* increased and dominated toward the middle of the fermentation process.

Saccharomycopsis fibuligera, the most abundant yeast species detected in the traditional wine starter of *Hong Qu* and *nuruk*, maintains a strong saccharification capability and produces various enzymes, particularly α-amylase, glucoamylase, acid proteases, and β-glucosidase (62). In addition, it can metabolize the native starch into maltose, dextrin, and glucose, thus indicating this species may play an important role during the initial stage of alcoholic fermentation.

***Monascus purpureus* pigment** Safety concerns appeared with the growing application of synthetic coloring agents, which resulted in the demand for natural food colorants. Natural food colorants are typically derived from the raw materials acquired from flowering plants, microorganisms, and insects (63,64). Therefore, the fungal production of natural food coloring offers an organic potential resource in relation to the existing natural colorant production (65). Fungal pigments are produced as secondary metabolites, known as polyketides. The polyketide pigments of commercially available *Monascus* have been used as food colorants for hundreds of years in Asia (66,67). The fungal *Monascus* pigments (Fig. 4) have been well researched and reviewed in relation to their structures, biosynthetic pathway, fermentation processes, physicochemical properties, detection methods, functions, and molecular biological activity (21). Furthermore, Mapari *et al*. (68) reviewed the production of natural food colorants, including *Monascus* pigments.

Monascus fungi produce at least six major related pigments, which can be classified into three groups based on color: (1) yellow pigments: monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$), (2) orange pigments: monascorubin ($C_{23}H_{26}O_5$) and rubropunctain ($C_{21}H_{22}O_5$), and (3) red pigments: monascorubramine ($C_{23}H_{27}NO_4$) and rubropunctamine ($C_{21}H_{23}NO_4$). Currently, more than 50 MPs have been identified. The yellow, orange, and red pigments of *Monascus* spp. can be detected by a spectrophotometer at 400, 470, and 500 nm, respectively.

The *Monascus* pigments biosynthesis is considered to generally follow a polyketide pathway; however, the *Monascus* pigments biosynthesis pathway is still unclear, somewhat controversial, and in need of scientific clarification. A possible biosynthetic pathway of monascin and monascusones A and B was proposed. The interconversion among the three types (yellow, orange, and red) of MPs compounds proposed that only orange pigment components (rubropunctatin and onascorubrin) were biosynthetic and the others were transformed from them by chemical transformations (69).

Monascus red pigment overproduction has been intensively investigated by various processes (70-72). Solid-state fermentation (73) and liquid-state fermentation (74) are two major processes for *Monascus* pigment production (75). The color characteristics and structures of the pigments that are produced by *Monascus* fermentation with various amino acids have also been studied (76,77). When each amino acid is included into the fermentation broth as a precursor, pigment extracts with different hues and color values are achieved,

depending on the specific content ratios of yellow, orange, and red colors within the fermentation broth. The yellow color and orange pigments are indistinguishable, regardless of the amino acid addition. The red compounds vary, according to the type of amino acid added. LC-MS and ^{1}H and ^{13}C NMR structural analyses verified that the derivative pigments contain the moieties of the added amino acids. The nitrogen source is considered an important regulatory factor; moreover, the effect of nitrogen sources has also been investigated. Nitrate and organic nitrogen sources, such as monosodium glutamate, amino acids, or yeast extract, support the formation of red pigmentation (78). Wang *et al*. (79) investigated the relationship between lipid and *Monascus* pigment accumulation by extractive fermentation. Rice medium, with the pH controlled at 3.0, results in the appearance of yellow pigments; rice medium with a neutral pH of 7 results in the appearance of red pigments (80).

Furthermore, the photostability of the pigments has been examined under various physical and chemical conditions (77,81,82). Under sunlight, the half-life of derivatives increases to 1.45-5.58 h, corresponding to a 6-25-fold improvement over a control red pigment (0.22 h). Pigment stability under UV light (365 nm) shows a pattern similar to that after exposure to sunlight. The differences in the degradation patterns revealed that the control red slowly changes to brown, whereas the phenylalanine derivative remains a weak red (76).

Monascus spp. pigments, as natural food colorant, have been widely utilized in the food industry, especially in meat products. Pattanagul *et al.* (20) applied anka as a red pigment to improve the color of meat sausages. Shehata *et al*. (83) studied natural colorants used in fresh Egyptian beef sausage. The consumers favored sausages with the addition of both *Monascus* spp. pigment and nitrite, which were added to improve natural color stability.

Health benefits of Hong Qu: pharmacological effects of *Monascus fermentate* Red yeast rice has been used for centuries in China as medicine and as a food source. The red yeast rice has been studied to lower cholesterol, improve blood circulation, and aid in abating digestive problems. Red yeast rice contains chemicals that correspond to prescription statin medications (84-86). One of these is monacolin K, which is a statin-like chemical (87). Lovastatin (formerly called Monacolin K), a specific and strong competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), is a powerful serum cholesterol-lowering drug in humans (88,89). Thus, a few studies have indicated that red yeast rice actually lowers LDL cholesterol levels and exhibits anticancer properties, which may effectively attack and prevent human gastric carcinoma cells (90). Red yeast inhibits the action of an enzyme in the body that aids in the production of cholesterol (91,92); however, the amount of Monacolin in red rice is less than that contained in the prescription; therefore, there may be other compounds in red yeast rice that lower cholesterol. On the other hand, one of the recent trends is the treatment of bone fracture by using lovastatin. Lovastatin stimulates bone formation *in vitro* and *in vivo*; when given in large doses or by prolonged infusions, it stimulates the biomechanical strength of murine long bones with healing fractures (88).

In contrast, red yeast rice may contain citrinin, another secondary metabolite known as a hepato-nephrotoxic mycotoxin for humans, which was found to be synthesized by *Monascus* strains (93). It was reported that citrinin could cause cell death to human embryonic kidney cells in a range of 1.8-4.7 mg/mL (94). According to animal tests, it was proposed that less than 2 ppm citrinin in *Monascus*-fermented products might be a safe concentration (95,96). However, *Hong Qu* rice wine has been used as traditional rice wine without a reported or known case of undesirable effects because of the low concentrations in the rice wine products.

The fungal *Monascus* pigments produced as the secondary metabolites are also considered to possess a number of health benefits. The red pigments, rubropunctamine and monascorubramine, show strong cytotoxicity and antimitotic effects on IHKE (immortalized human kidney epithelial) cells (22). The orange pigments, monascorubrin and rubropunctatin, have been found to have antibiotic activity against bacteria, yeast, and filamentous fungi (23,24) and inhibit the growth of tumors (25). Yellow pigments, including monascin and ankaflavin, have displayed anti-inflammation activity (26,27), cancer cell cytotoxic activity (97,98), antihypertensive activity (26), and cholesterol-lowering activity (99). Monascin was also reported to have a therapeutic potential, as related to diabetes and diabetic-associated oxidative stress complications (100).

Conclusion

Traditional rice wine making technology, such as that used for making *Hong Qu* rice wine, have been used in China for more than 10 centuries and similar to those of *nuruk* and *makkoli* in Korea and many other countries in Asia. The production of traditionally fermented rice wines and starters are empirical and take place in homes and villages under undefined conditions. The starter cultures are often handed down from generation to generation by way of serial re-culturing and are associated with the geographical environments, which also play a significant role in preparing the starter cultures, as climatic conditions are highly variable from country to country, resulting in a large amount of regional diversity. Thus, studies have failed to establish any pattern within microbiology. Comparison studies across the countries can provide an insight into understanding the microbial diversity of various fermentation products. On the other hand, the classical analytical techniques, i.e., the culture-dependent methods, may miss an entire species. Recent developments of culture-independent DNA extraction methods are a promising tool for accurately identifying microbial species.

However, further studies are desirable in the international standardization of microbe and manufacturing processes. In the future, it may be possible to secure an efficient and controlled

process of rice wine making in selected, safe microbial strains, combined with molecular biotechnology. Future research should include the metabolic pathway study of secondary metabolites, such as pigments and flavor compounds, from microorganisms.

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