RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2017, **7**, 44766

Identification and characterization of epiphytic yeasts on apples in China

Jianping Wei, Chen Niu, Bin Liu, Yahong Yuan and Tianli Yue *

Currently, little is known regarding the prevalence and diversity of epiphytic yeasts on apples. Therefore, in this study we attempted to identify and characterize yeasts on apples for their potential cider-making performance. A total of 754 yeast strains were isolated from the skins of apples collected from eight provinces in the two main ecological regions of China, *i.e.*, the Loess Plateau and Bo Hai Gulf regions. More than 71 species belonging to 24 genera were identified and *Aureobasidium* (*A.*) *pullulans* and *Hanseniaspora* (*H.*) *uvarum* were the most predominant yeast species. We did not observe any *Saccharomyces cerevisiae* strains on the associated apple samples. Correspondence analysis revealed the following connection between yeast genera and the provinces: *Hanseniaspora* in Shaanxi, *Rhodotorula* in Liaoning, *Candida* in Shandong, *Aureobasidium* in Shanxi and *Pichia* in Hebei. No dominant genera were observed in apples from Gansu, Henan and Ningxia. Although genetic differences among the 23 *H. uvarum* strains were identified, there was no clear link between the isolates as to origin, genotype and phenotype following RAPD-PCR analysis and some qualitative evaluation of the yeast biochemical activities.

Received 26th July 2017
Accepted 10th September 2017

DOI: 10.1039/c7ra08234g

rsc.li/rsc-advances

1. Introduction

Fruit surfaces, the natural reservoirs for sugars and other nutrients, are more or less colonized by different yeast species. Studies have shown that apart from interfering with apple health and quality,[1] epiphytic yeasts can affect the safe consumption and flavour of both apples and cider.[2–5] Yeast species are crucial determinants of several important characteristics associated with some fermented products, including wine,[6,7] and orange wine.[8]

Despite their importance, the diversity of epiphytic yeasts on apples has yet to be elucidated. Thus, studies to identify and characterize yeasts that naturally reside on apples are required to fully understand the potential role of these yeasts in food processing applications. Indeed, a large body of research has been performed in respect of grape berry surface yeast characterization[9,10] and assays utilized in these studies can also be used for the characterization of microorganisms on other fruit surfaces.

China has four major apple-producing districts, each with distinct geographical and climatic characteristics. As the biggest global apple-producing country over the last few decades,[11] China has the potential to take full advantage of indigenous yeast species to develop its cider industry. However, information pertaining to the microbiota of fruits from specific regions is limited. Thus, more research should be performed to determine the effects of yeast community composition on regional cider production.

College of Food Science and Engineering, Northwest A&F University, 712100 Yangling, Shaanxi, China. E-mail: yuetl305@nwsuaf.edu.cn; Tel: +86-29-87092492

Random amplified polymorphism DNA-PCR (RAPD-PCR) is a kind of fingerprint technique for differentiation of the isolates at the subspecies level. RAPD-PCR has been widely utilized in yeast classification due to its rapidity and sensitivity. Indeed, this approach has been used to assess interspecific and intraspecific diversity of different yeasts such as *Kluyveromyces marxianus*,[12] *Candida zemplinina*,[13] and *Pichia fermentans*.[14]

The aim of this study was to investigate the prevalence and diversity of indigenous culturable yeasts and yeast-like fungi (referred to here as yeasts) on apples from the two main cultivated districts in China. Furthermore, the impact of geographic location on yeast distribution was evaluated. For some of the selected isolates, intraspecific genetic variations were evaluated by RAPD analysis. The study also investigated diverse technological properties with industrial applications.

2. Materials and methods

2.1 Apple sampling

A total of 119 apple samples grown at 43 sampling sites (Fig. 1 and Table 1) from the Loess plateau and Bo Hai Gulf Area regions of China were analysed as part of this study. Healthy and undamaged apples were collected in aseptic bags from orchards. These apples were placed in portable refrigerators prior to arrival in the laboratory.

2.2 Yeast enumeration and isolation

Apple samples were aseptically peeled and mixed. Samples (approximately 10 g each) were placed in a flask with 90 mL of sterile normal saline. The flasks were subsequently shaken at

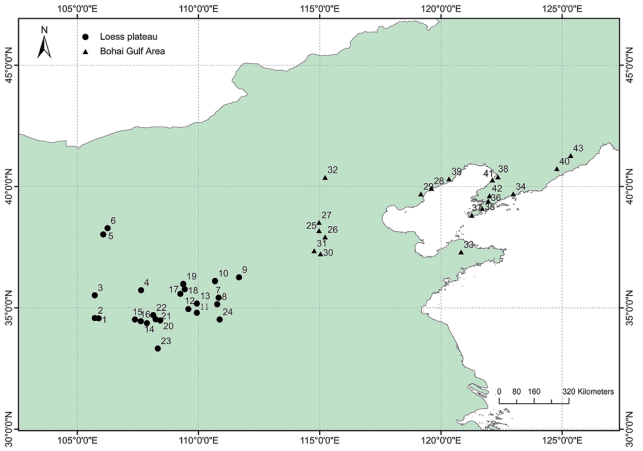

Fig. 1 Map of sampling sites in China.

120 rpm for 30 minutes to elute microorganisms. Next, 100 μL aliquots (taken from the flasks) were serially diluted and spread onto YPD agar containing 100 mg L^{-1} chloramphenicol to inhibit bacterial growth.[15] After incubation at 28 °C for 48–72 h, the total number of colonies on each plate was counted. All experiments were performed in duplicate. Ten colonies from each plate were randomly selected for purification. Alternatively, all colonies were selected if the total amount of colonies on the plate was less than 10 (revised from the procedure published by Osorio-Cadavid *et al.*[16]). After streaking to purification, strains were preserved at −80 °C in 20% (v/v) glycerol.

2.3 DNA extraction

A Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd.) was used in conjunction with lyticase (Solarbio) to extract DNA according to the manufacturer's protocol.

2.4 Sequencing of the 26S rRNA gene (S) D1/D2 domain

The large-subunit (LSU) D1/D2 domain was amplified and sequenced with primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG) and NL4 (5′-GGTCCGTGTTTCAAGACGG). Each PCR mixture (total volume of 50 μL) contained 3 μL of DNA template, 25 μL of premix Taq polymerase (TAKARA, China), and 1 μL of each primer (10 μmol L^{-1}). The PCR conditions were as follows: 94 °C for 3 min followed by 36 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, and a final extension step at 72 °C for 5 min. PCR products were analyzed following gel electrophoresis on a 1.5% agarose gel at 110 V for approximately 40 min. Extracted PCR products were sent to Shanghai Thermo Fisher Scientific Inc. for purification and sequencing. The sequences that were obtained for each strain were searched and compared using Blast and the NCBI database (http://www.ncbi.nlm.nih.gov/blast).

2.5 Frequency percentage analysis

In order to determine the most prevalent yeast genera, frequency percentage analysis was performed. Colonies were randomly isolated from the highest dilution plates. This approach was most likely to select for strains that belonged to the more dominant species.[17] Species distribution in the samples was subsequently studied according to a previously published method.[18] This method permits calculation of the number of times each species was detected as opposed to the number of strains. Using this approach, we can determine the number of positive samples for each species and the corresponding frequency. The frequency was defined as the number of positive samples of a species divided by the total number of samples (expressed as a percentage).

2.6 RAPD-PCR

Twenty-two isolates of *H. uvarum*, the most abundant isolated yeast species, were subjected to RAPD-PCR analysis using primer P24 (5′-GCGTGACTTG-3′).[19] ACCC20310 (a *Hanseniaspora uvarum* type strain) was added and used as an internal control during this analysis. Genomic DNA was extracted as previously described. The PCR system and procedure are identical to those described by Chen *et al.*[20] The DL2000 DNA marker was used to confirm the sizes of the associated products after electrophoresis on 2% agarose gels in 1× TAE buffer at 70 V for 90 min. The results from at least two independent experiments were used in this analysis. The bands were manually identified and allocated one of two codes (0 or 1) depending on whether the corresponding bands were present or not. The resultant matrix was used to generate a phonogram based on the unweighted pair group method and the arithmetic mean algorithm (UPGMA) method using NTsys software (version 2.10e; Exeter Software, New York, NY).

2.7 Qualitative evaluation of yeast biochemical activities

Different assays were performed to rapidly evaluate the activities of secreted enzymes (including protease, β-glucosidase, catalase, xylanase and esterase) of enological relevance. Twenty-two selected yeasts (as mentioned in Section 2.6) were grown on YPD plates prior to testing and experiments were performed in triplicate.

A method described by Englezos *et al.*[21] was used to evaluate the ability of different yeast species to produce extracellular proteases. An equal volume of skim milk solution (final concentration of 10 g L^{-1}) and a mixture were placed in separate flasks prior to agar plate preparation. The mixture contained 3 g L^{-1} malt extract, 3 g L^{-1} yeast extract, 5 g L^{-1} bacteriological peptone, 10 g L^{-1} glucose, 5 g L^{-1} NaCl and 20 g L^{-1} agar. The two media were mixed and the pH of the mixture was adjusted to pH 3.5 (using 0.1 M HCl) prior to pouring on sterile Petri dishes. The isolates were spot-inoculated and then incubated at 25 °C for 3 days. A clear zone around the yeast colonies signified protease activity.

Esterase production was assessed by replica plating the yeast isolates onto media containing 1% bacteriological peptone, 0.5% NaCl, 0.01% $CaCl_2$ and 1.5% agar. After autoclaving, the medium was cooled to about 50 °C and 5 mL of sterile Tween 80 was added to 1 L of medium. The agar plates were spot-inoculated and then incubated at 30 °C for 2 days.[21] Colonies showing a visible opaque halo were identified as positive.

β-Glucosidase, catalase and xylanase activities were determined using a previously published procedure.[22]

Table 1 Sample sites, coordinates, number of samples and corresponding codes

No.	Province	Coordinates	N^a	Code
1	Gansu	N34.57, E105.88	3	G1, G2, G3
2	Gansu	N34.58, E105.72	1	G4
3	Gansu	N35.52, E105.72	3	G5, G6, G7
4	Gansu	N35.73, E107.63	3	G8, G9, G10
5	Ningxia	N38.02, E106.07	4	N1, N2, N3, N4
6	Ningxia	N38.28, E106.25	4	N5, N6, N7, N8
7	Shanxi	N35.42, E110.83	3	T1, T2, T3
8	Shanxi	N35.15, E110.77	3	T4, T5, T6
9	Shanxi	N36.25, E111.67	5	T7, T8, T9, T10, T11
10	Shanxi	N36.10, E110.68	1	T12
11	Shaanxi	N34.80, E109.93	4	X1, X2, X3, X4
12	Shaanxi	N34.95, E109.58	1	X5
13	Shaanxi	N35.18, E109.93	6	X6, X7, X8, X9, X10, X11
14	Shaanxi	N34.37, E107.87	2	X12, X13
15	Shaanxi	N34.52, E107.38	2	X14, X15
16	Shaanxi	N34.45, E107.62	2	X16, X17
17	Shaanxi	N35.58, E109.25	1	X18
18	Shaanxi	N35.77, E109.43	1	X19
19	Shaanxi	N35.98, E109.37	1	X20
20	Shaanxi	N34.48, E108.42	7	X21, X22, X23, X24, X25, X26, X27
21	Shaanxi	N34.53, E108.23	4	X28, X29, X30, X31
22	Shaanxi	N34.70, E108.13	1	X32
23	Henan	N33.32, E108.32	4	Y1, Y2, Y3, Y4
24	Henan	N34.52, E110.87	6	Y5, Y6, Y13, Y14, Y15, Y16
25	Hebei	N38.18, E114.97	3	J1, J2, J3
26	Hebei	N37.92, E115.22	1	J4
27	Hebei	N38.52, E114.97	1	J5
28	Hebei	N39.93, E119.60	4	J6, J7, J8, J9
29	Hebei	N39.70, E119.17	2	J10, J11
30	Hebei	N37.22, E115.03	1	J12
31	Hebei	N37.35, E114.77	2	J13, J14
32	Hebei	N40.38, E115.22	3	J15, J16, J17
33	Shandong	N37.30, E120.83	6	L1, L2, L3, L8, L9, L10
34	Liaoning	N39.70, E122.98	7	S1, S11, S13, S15, S16, S21, S23
35	Liaoning	N39.10, E121.70	1	S2
36	Liaoning	N39.40, E121.95	1	S3
37	Liaoning	N38.82, E121.27	1	S4
38	Liaoning	N40.40, E122.35	5	S5, S7, S8, S9, S17
39	Liaoning	N40.32, E120.33	1	S6
40	Liaoning	N40.73, E124.78	4	S10, S12, S14, S18
41	Liaoning	N40.27, E122.12	2	S19, S22
42	Liaoning	N39.62, E122.00	1	S20
43	Liaoning	N41.27, E125.35	1	S24

[a] Number of samples.

2.8 Statistical analysis

Significant differences between the mean of the number of isolates from eight provinces were compared using Duncan's multiple range test at 95% probability level. Data pertaining to the main genera were subjected to correspondence analysis to explore the effect of geographic location on yeast diversity. All of these analyses were performed using the IBM SPSS Statistics (version 22.0, IBM Corp., Armonk, NY, USA) software package.

3. Results and discussion

3.1 Yeast loads on apples

A total of 754 yeast and yeast-like strains (referred to here as yeasts) were isolated from the surfaces of 18 apple species grown at 43 sites in eight provinces in the Loess Plateau and the Bo Hai Gulf Area regions of China. We evaluated the total yeast population using the spread plate technique on YPD medium. The yeast populations that were observed on the apple sample surfaces are shown in Fig. 2. The yeast loads on apple samples varied between 10^3 and 10^5 CFU g^{-1}. These values approximate with data obtained from a previous report pertaining to sound grapes in China.[23] However, for some reason we did not observe any yeast isolates on samples T4, X1, X7, and Y1. Obviously, total counts of samples were relatively high in Shandong and low in Henan province, respectively, while data obtained from other provinces were not statistically different (5%).

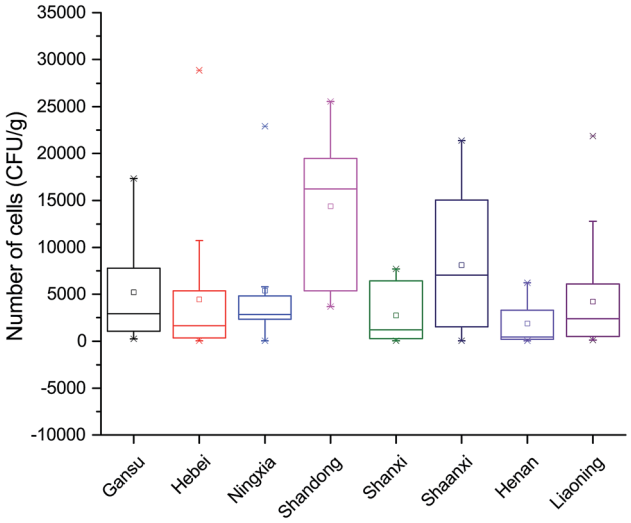

Fig. 2 Box plots of positive sample microbial counts on YPD media.

3.2 Molecular identification and frequency percentage analysis

The yeast communities on samples were both complex and diverse. More than 71 different yeast species belonging to 24 different genera were observed following sequencing of the 26S rRNA gene (S) D1/D2 domain. Table 2 shows the frequency of positive samples pertaining to the different species. The composition of indigenous yeast species on apples differed among the eight provinces sampled. *Aureobasidium* (*A.*) *pullulans* and *Hanseniaspora* (*H.*) *uvarum* were the most common species, being isolated in 52.94% and 29.41% of total samples, respectively. The next most prevalent species were *Rhodotorula* (*R.*) *glutinis* (16.81%), *Cryptococcus* (*C.*) *flavescens* (14.29%), *Pichia* (*P.*) *guilliermondii* (13.45%) and *Pichia* (*P.*) *kluyveri* (10.92%). A total of 33 species were found in a single sample only. Interestingly, we did not observe any *Saccharomyces* (*S.*) *cerevisiae* isolates. *A. pullulans* and *H. uvarum* represented 27.06% and 22.55% of the 754 isolates, respectively, suggesting that these species were the dominant species in our samples.

A. pullulans is predominantly distributed on plant surfaces and is a polymorphic fungus with a complex life cycle. This fungal species is known for its ability to produce melanin and has also been reported to secrete large amounts of extracellular enzymes for biotechnological applications.[24] A previous study observed that following treatment with β-glucosidases produced by *A. pullulans*, the amount of monoterpenes in wine increased significantly.[25] It has also been suggested that cold-active pectinases from *A. pullulans* could be used to optimize wine production,[26] while some scientists speculate that treatment with these enzymes may indirectly improve the aroma and flavour of cider. *A. pullulans* has also been utilized as a biocontrol product for different apple-specific pathogens (*Botrytis cinerea*, *Monilinia fructigena*, *Penicillium expansum*, and *Pezicula malicorticis*).[27]

H. uvarum represents the teleomorphic form of *Kloeckera apiculata*, and is a widely distributed yeast species that is often isolated from the environment, the initial stages of natural fermentations and industrial food production processes. Many researchers have observed that this apiculate yeast species can dominate fruit skin microbial populations.[8,28,29] However, Grangeteau[30] reported that genus *Hanseniaspora* represented 28% of the isolates and was not isolated for 2 consecutive years, respectively. The reasons for these observations are not completely understood and further research is required to elucidate these findings. It is well known that yeast species exhibit both beneficial and detrimental effects concerning both food and drink. For instance, yeast is known to cause spoilage during orange juice production.[31] In addition, yeast species have been isolated from cider[32] and other fermented products including wine[33] and table olives.[34] There have been several attempts to assess the oenological potential of *H. uvarum* in simultaneous fermentation and as a mixed starter culture with *S. cerevisiae*. The results of these efforts suggest that *H. uvarum* may elicit beneficial effects during winemaking.[35,36] However, some of the characteristics that were observed for *H. uvarum* were found to be strain-dependent.[37] Therefore, it is necessary to determine whether all isolates are capable of contributing to quality improvement during cider production.

The prevalence of other species of yeasts (apart from *H. uvarum*) was relatively low (<20%) on the fruit surfaces. Microorganisms usually occupy plant surfaces by accident and associated populations are usually wiped out before long if not replenished.[38] However, some yeast species exhibit favorable traits regarding brewing, even when present in reduced quantities, especially when the brewing is coordinated with *S. cerevisiae*.[39] Indeed, yeast species including *Kluyveromyces thermotolerans*, *Metschnikowia pulcherrima* and *Torulaspora delbrueckii* are commercially available due to their beneficial characteristics in this regard.[40] However, further research needs to be performed in order to fully validate the use of these species for commercial applications.

Variable results have been generated with regard to the prevalence of *S. cerevisiae* in orchard samples. This variability has resulted in contention regarding the appropriateness of the approaches that were utilized to perform these analyses. Several studies have suggested that the use of the direct agar plating method rather than an enrichment culture strategy might explain why *S. cerevisiae* isolates were infrequently observed in samples.[41–43] Interestingly, researchers have managed to isolate *S. cerevisiae* from wine samples.[28,44] Thus, it would appear that *S. cerevisiae* isolates are easier to screen following fermentation. In this study, we did not observe any yeast isolates from the genus *Saccharomyces*. It has been reported that *S. cerevisiae* occurs on one out of every 1000 fruit surfaces analysed.[45] Therefore, it is likely that we would have observed this species if a greater number of samples were gathered.

3.3 Correspondence analysis

Bacterial and fungal prevalence and distribution on fruit associated with different geographic locations was studied as part of this analysis.[46,47] Results showed that geographic factors could influence heterogeneity of microbiological community. In our

Table 2 Number of positive samples for each yeast species

Species	Ps[a]	Frequency
Aureobasidium pullulans	63	52.94%
Hanseniaspora uvarum	35	29.41%
Rhodotorula glutinis	20	16.81%
Cryptococcus flavescens	17	14.29%
Pichia guilliermondii	16	13.45%
Pichia kluyveri	13	10.92%
Candida fermentati	11	9.24%
Cryptococcus albidus	10	8.40%
Rhodotorula graminis	9	7.56%
Debaryomyces hansenii	8	6.72%
Hanseniaspora vineae	8	6.72%
Candida zemplinina	7	5.88%
Filobasidium sp.	6	5.04%
Cryptococcus aureus	5	4.20%
Cryptococcus tephrensis	5	4.20%
Cryptococcus victoriae	5	4.20%
Torulaspora delbrueckii	4	3.36%
Bulleromyces albus	3	2.52%
Candida sorbosivorans	3	2.52%
Candida zeylanoides	3	2.52%
Cryptococcus saitoi	3	2.52%
Metschnikowia pulcherrima	3	2.52%
Metschnikowia sp.	3	2.52%
Pseudozyma aphidis	3	2.52%
Sporidiobolus pararoseus	3	2.52%
Candida melibiosica	2	1.68%
Candida metapsilosis	2	1.68%
Candida oleophila	2	1.68%
Candida parapsilosis	2	1.68%
Candida sake	2	1.68%
Candida sp.	2	1.68%
Cryptococcus sp.	2	1.68%
Cryptococcus terrestris	2	1.68%
Cryptococcus uzbekistanensis	2	1.68%
Hanseniaspora opuntiae	2	1.68%
Hanseniaspora osmophila	2	1.68%
Rhodotorula mucilaginosa	2	1.68%
Rhodotorula sp.	2	1.68%
Candida catenulata	1	0.84%
Candida corydali	1	0.84%
Candida quercitrusa	1	0.84%
Candida stellata	1	0.84%
Cryptococcus albidosimilis	1	0.84%
Cryptococcus cf. *taibaiensis*	1	0.84%
Cryptococcus heveanensis	1	0.84%
Cryptococcus luteolus	1	0.84%
Cystofilobasidium ferigula	1	0.84%
Filobasidium elegans	1	0.84%
Filobasidium uniguttulatum	1	0.84%
Hannaella sinensis	1	0.84%
Hannaella zeae	1	0.84%
Hyphopichia burtonii	1	0.84%
Issatchenkia occidentalis	1	0.84%
Issatchenkia terricola	1	0.84%
Lachancea thermotolerans	1	0.84%
Meira argovae	1	0.84%
Meira cf. *geulakonigii*	1	0.84%
Metschnikowia aff. *fructicola*	1	0.84%
Pichia kudriavzevii	1	0.84%
Pichia manshurica	1	0.84%
Pichia nakasei	1	0.84%
Pichia scaptomyzae	1	0.84%
Rhodosporidium fluviale	1	0.84%
Rhodosporidium sp.	1	0.84%
Rhodotorula ingeniosa	1	0.84%
Rhodotorula nothofagi	1	0.84%
Sarocladium kiliense	1	0.84%
Saturnispora silvae	1	0.84%
Sterigmatomyces elviae	1	0.84%
Symmetrospora sp.	1	0.84%
Torulaspora quercuum	1	0.84%

[a] Positive samples.

study, six genera including *Aureobasidium*, *Candida*, *Cryptococcus*, *Hanseniaspora*, *Pichia*, and *Rhodotorula* were isolated in samples from at least seven provinces. These genera accounted for 90.05% of the total isolates identified. A Correspondence Analysis (CA) (Fig. 3) was carried out using these six genera and the provinces from which the samples were isolated. Following analysis of these results, the yeasts that proved to be characteristic of the different provinces were as follows: *Hanseniaspora* in Shaanxi, *Rhodotorula* in Liaoning, *Candida* in Shandong, *Aureobasidium* in Shanxi and *Pichia* in Hebei. There was no obvious correlation between any yeast genera and Gansu, Henan and Ningxia.

These results demonstrate that geographic location associates with both the density and diversity of the observed yeast genera. Thus, it is possible that geographic location can influence the microbiota associated with spontaneous fermentations, thereby promoting the production of regional cider with specialized characteristics. We hope to conduct future studies pertaining to the relationship between regionally occurring yeast species and cider flavour.

3.4 RAPD-PCR analysis

H. uvarum has been shown to elicit beneficial effects in winemaking.[33] However, intraspecific variability in relation to these phenomena needs to be further studied.

Among the 170 *H. uvarum* strains that were isolated from the eight provinces, 22 isolates (including three isolates from each province except Ningxia) were randomly selected for strain diversity identification (ACCC20310 was added as an internal control). The UPGMA dendrogram based on the RAPD profile is shown in Fig. 4. The dendrogram (although origin-nonspecific) demonstrates that there are some differences among these isolates.

3.5 Yeast biochemical properties

Despite the non-specific nature of this analysis (with respect to geographic location), we investigated whether genetic diversity correlates with phenotypic differences. Due to the fact that phenotyping assays can be relatively time-consuming, 22 representative isolates were qualitatively characterized using various plate assays. At the phenotypic level, several

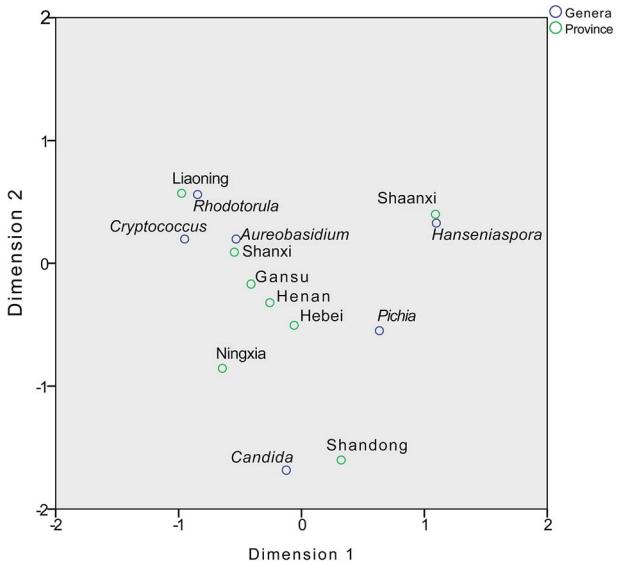

Fig. 3 Correspondence analysis (CA) of main yeast genera from eight provinces.

extracellular enzymatic activities of ecological relevance (protease, β-glucosidase, catalase, xylanase and esterase) were assayed; however, we did not observe any significant diversity in connection with the associated activities. All strains exhibited protease and catalase activity, while no β-glucosidase, xylanase, or esterase activities were observed. Thus, genotypic differences did not correlate with variations in phenotypes. These results are in agreement with a previous study conducted by Albertin *et al.*,[48] but differ from observations made in a separate report.[37]

It should be stated that our analyses were conducted following growth of *H. uvarum* in artificial media. Several reports have shown that yeast was strain-specific in many aspects of wine-making.[35,49,50] Isolates can be distinguished at the fermentation level and subsequently screened for cider-making.

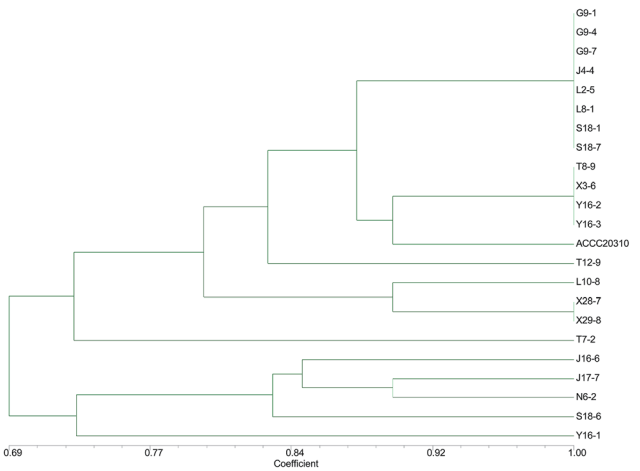

Fig. 4 Dendrogram of RAPD patterns showing the relatedness of *H. uvarum* isolates from different provinces.

4. Conclusions

In conclusion, this study provides the first report regarding yeast diversity on apples in the Loess Plateau and Bo Hai Gulf regions of China. These results suggest that apple-associated microbial diversity is non-randomly associated with the apple farming regions from which the apples were procured. We did not observe a clear association between geographic origins, yeast isolate genotype and associated phenotypes. Therefore, further studies are required to determine whether these regionally differential yeasts actually influence the organoleptic and chemosensory perception of cider.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors acknowledge the financial support of National Basic Research Program of China (2013FY113400) and National Natural Science Foundation of China (31371814, 31671866).

Notes and references

1 A. Graça, D. Santo, E. Esteves, C. Nunes, M. Abadias and C. Quintas, *Food Microbiol.*, 2015, **51**, 179–185.
2 R. R. Madrera, A. G. Hevia, N. P. García and B. S. Valles, *LWT–Food Sci. Technol.*, 2008, **41**, 2064–2069.
3 Z. Liang, Z. Cheng and G. S. Mittal, *LWT–Food Sci. Technol.*, 2006, **39**, 351–357.
4 M. Gouma, E. Gayán, J. Raso, S. Condón and I. Álvarez, *Innovative Food Sci. Emerging Technol.*, 2015, **32**, 146–155.
5 A. A. Gabriel, *Ultrason. Sonochem.*, 2012, **19**, 346–351.
6 C. Garofalo, M. El Khoury, P. Lucas, M. Bely, P. Russo, G. Spano and V. Capozzi, *J. Appl. Microbiol.*, 2015, **118**, 1395–1408.
7 S. M. D. Mónaco, M. E. Rodríguez and C. A. Lopes, *Int. J. Food Microbiol.*, 2016, **230**, 31–39.
8 R. Liu, Q. Zhang, F. Chen and X. Zhang, *Ann. Microbiol.*, 2015, **65**, 1–5.
9 R. Vincent, C. Olivier and L. F. Aline, *Aust. J. Grape Wine Res.*, 2005, **11**, 316–327.
10 P. Raspor, D. M. Milek, J. Polanc, S. S. Mozina and N. Cadez, *Int. J. Food Microbiol.*, 2006, **109**, 97–102.
11 WAPA (The World Apple and Pear Association), World apple production 2003–2013 in T (FAOstat) 2015, http://www.wapa-association.org/asp/page_1.asp?doc_id=446.
12 R. Tofalo, G. Fasoli, M. Schirone, G. Perpetuini, A. Pepe, A. Corsetti and G. Suzzi, *Int. J. Food Microbiol.*, 2014, **187**, 41–49.
13 W. P. Pfliegler, E. Horváth, Z. Kállai and M. Sipiczki, *Microbiol. Res.*, 2014, **169**, 402–410.
14 L. Caputo, L. Quintieri, F. Baruzzi, M. Borcakli and M. Morea, *Food Res. Int.*, 2012, **48**, 755–762.

15 G. V. D. M. Pereira, V. T. Soccol, A. Pandey, A. B. P. Medeiros, A. L. Gollo and C. R. Soccol, *Int. J. Food Microbiol.*, 2014, **188**, 60–66.
16 E. Osorio-Cadavid, C. Chaves-López, R. Tofalo, A. Paparella and G. Suzzi, *Food Microbiol.*, 2008, **25**, 771–777.
17 A. Pulvirenti, L. Solieri, M. Gullo, L. D. Vero and P. Giudici, *Lett. Appl. Microbiol.*, 2004, **38**, 113–117.
18 L. Solieri, S. V. Landi, L. De Vero and P. Giudici, *J. Appl. Microbiol.*, 2006, **101**, 63–71.
19 C. Pina, P. Teixeiró, P. Leite, M. Villa, C. Belloch and L. Brito, *J. Appl. Microbiol.*, 2005, **98**, 1107–1114.
20 N. Chen, Y. Yuan, Z. Hu, Z. Wang, B. Liu, H. Wang and T. Yue, *Int. J. Food Microbiol.*, 2016, **232**, 126–133.
21 V. Englezos, K. Rantsiou, F. Torchio, L. Rolle, V. Gerbi and L. Cocolin, *Int. J. Food Microbiol.*, 2015, **199**, 33–40.
22 J. Bautista-Gallego, F. Rodríguez-Gómez, E. Barrio, A. Querol, A. Garrido-Fernández and F. N. Arroyo-López, *Int. J. Food Microbiol.*, 2011, **147**, 89–96.
23 S. S. Li, C. Chao, L. Zheng, J. Y. Chen, B. Yan, B. Z. Han and M. Reeves, *Int. J. Food Microbiol.*, 2010, **138**, 85–90.
24 C. Gostinčar, R. A. Ohm, T. Kogej, S. Sonjak, M. Turk, J. Zajc, P. Zalar, M. Grube, S. Hui and J. Han, *BMC Genom.*, 2014, **15**, 1–29.
25 M. A. Baffi, T. Tobal, J. H. G. Lago, M. Boscolo, E. Gomes and R. Da-Silva, *Appl. Biochem. Biotechnol.*, 2013, **169**, 493–501.
26 M. E. Farías and V. M. D. Ambrosini, *Int. J. Food Microbiol.*, 2011, **147**, 144–148.
27 A. Wagner, B. Hetman, M. Kopacki and A. Jamiołkowska, *Acta Agrobot.*, 2013, **66**, 77–88.
28 A. Maria and B. Giuseppe, *Front Microbiol.*, 2016, **7**, 00809.
29 M. Brysch-Herzberg and M. Seidel, *Int. J. Food Microbiol.*, 2015, **214**, 137–144.
30 C. Grangeteau, D. Gerhards, S. Rousseaux, C. V. Wallbrunn, H. Alexandre and M. Guilloux-Benatier, *Food Microbiol.*, 2015, **50**, 70–77.
31 A. Renard, P. G. D. Marco, M. Egea-Cortines and J. Weiss, *Int. J. Food Microbiol.*, 2008, **126**, 195–201.
32 B. R. Pando, S. A. Querol and V. B. Suárez, *Food Microbiol.*, 2010, **27**, 503–508.
33 Y. A. Hong and H. D. Park, *Food Microbiol.*, 2013, **34**, 207–214.
34 H. Lucena-Padrós, B. Caballero-Guerrero, A. Maldonado-Barragán and J. L. Ruiz-Barba, *Food Microbiol.*, 2014, **42**, 154–165.
35 M. Tristezza, M. Tufariello, V. Capozzi, G. Spano, G. Mita and F. Grieco, *Front. Microbiol.*, 2016, **7**, 00670.
36 N. Moreira, C. Pina, F. Mendes, J. A. Couto, T. Hogg and I. Vasconcelos, *Food Contr.*, 2011, **22**, 662–667.
37 A. Capece, C. Fiore, A. Maraz and P. Romano, *J. Appl. Microbiol.*, 2005, **98**, 136–144.
38 T. Chand-Goyal and R. A. Spotts, *Microbiol. Res.*, 1996, **151**, 427–432.
39 P. T. Liu, L. Lu, C. Q. Duan and G. L. Yan, *LWT–Food Sci. Technol.*, 2016, **71**, 356–363.
40 N. P. Jolly, C. Varela and I. S. Pretorius, *FEMS Yeast Res.*, 2014, **14**, 688–689.
41 A. Barata, M. Malfeito-Ferreira and V. Loureiro, *Int. J. Food Microbiol.*, 2012, **154**, 152–161.
42 R. R. Davenport, *Vitis*, 1974, **13**, 123–130.
43 M. Stringini, F. Comitini, M. Taccari and M. Ciani, *Int. J. Food Microbiol.*, 2008, **127**, 184–189.
44 I. Vigentini, D. Maghradze, M. Petrozziello, F. Bonello, V. Mezzapelle, F. Valdetara, O. Failla and R. Foschino, *Front. Microbiol.*, 2016, **7**, 00352.
45 R. Mortimer and M. Polsinelli, *Res. Microbiol.*, 1999, **150**, 199–204.
46 M. D. C. Portillo, J. Franquès, I. Araque, C. Reguant and A. Bordons, *Int. J. Food Microbiol.*, 2015, **219**, 56–63.
47 N. A. Bokulich, J. H. Thorngate, P. M. Richardson and D. A. Mills, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 139–148.
48 W. Albertin, M. E. Setati, C. Miot-Sertier, T. T. Mostert, B. Colonna-Ceccaldi, J. Coulon, P. Girard, V. Moine, M. Pillet and F. Salin, *Front. Microbiol.*, 2014, **6**, 01569.
49 F. Gaensly, B. C. Agustini, G. A. D. Silva, G. Picheth and T. M. B. Bonfim, *J. Funct. Foods*, 2015, **19**, 288–295.
50 M. Paraggio, *Food Technol. Biotechnol.*, 2004, **42**, 165–168.

