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1 Evaluation of Chinese *Saccharomyces cerevisiae* wine strains from different
2 geographical origins

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25**Running Title:** Chinese *Saccharomyces* wine strains

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27**Key words:** wine; *Saccharomyces cerevisiae*; genetic diversity; interdelta sequence

28typing

Abstract: Interdelta sequence typing was used to investigate the genetic diversity of 54 Chinese indigenous wine strains of *S. cerevisiae* selected on the basis of preliminary sequence analysis from 349 strains that were isolated previously from 15 spontaneous fermentations in Shanshan, Xinjiang and Qing Tongxia, Ningxia, China. Of the 54 strains tested 78% (42/54) were confirmed as genetically distinct. Dendrograms based on strain similarity revealed differences in the genetic relationships of Xinjiang yeast populations between table and wine grape varieties in addition to differences between red and white grape varieties in Ningxia (Dice coefficients of 0.448 and 0.674, respectively). When data from *Saccharomyces* strains collected from California, France, Italy, Northern Europe, and Spain were included in the analysis, the dendrogram revealed 5 groups containing 51, 4, 48, 3, and 1 strain respectively. Ningxia and Xinjiang provinces displayed local specific *S. cerevisiae* biotas that show a clear separation from other strains. Cluster XJ19 isolated from Xinjiang displayed a high level of similarity with UCD587, UCD2515, and UCD2516 from California. Clusters XJ2, XJ7, XJ20, and XJ3 also isolated from Xinjiang had a lower degree of similarity with other Chinese indigenous genotypes and strains from other regions. This study compares, for the first time, the genetic diversity and relationships between indigenous *S. cerevisiae* wine strains collected from Xinjiang and Ningxia provinces in China with wine strains from different geographic regions.

49 *Saccharomyces cerevisiae* is an important experimental model organism in
50 addition to its commercial significance as the predominant yeast species during wine
51 fermentation. Modern strains of *S. cerevisiae* are thought to have arisen in Asia given
52 the diversity of strains and reproductive isolation observed in a study of *S. cerevisiae*
53 isolates from human-associated and non-human-associated environments in China
54 (Wang et al. 2012, Liti 2015). Distinct lineages were observed for isolates from
55 primeval and secondary forests (Wang et al. 2015). However this study considered
56 few isolates from wine environments and found fewer isolates of *S. cerevisiae* from
57 fruit sources and more from tree bark, rotting wood and soil samples than from fruit
58 samples. Of the fruits evaluated the fewest isolates were obtained from grapes (Wang
59 et al. 2012). The authors concluded that grape and orchard isolates were similar to
60 those of the wine European lineage. Our goal was to evaluate in greater depth the
61 diversity of natural vineyard isolates from two wine regions in China.

62 Several studies have reported on the genetic diversity of *S. cerevisiae* strains in
63 different wine-producing regions. These studies revealed that geographic region
64 (Versavaud et al. 1995, Goddard et al. 2010), climatic conditions (Valero et al. 2007),
65 vintage (Sabate et al. 1998, Mercado et al. 2007), grape varieties and must
66 characteristics (Blanco et al. 2012), inoculation of starter yeasts (Santamaria et al.
67 2005), and SO₂ addition (Wang and Liu 2013) affected the diversity of *S. cerevisiae*
68 observed. In many cases genetically distinct strains of *S. cerevisiae* were isolated from
69 the same fermentation during wine fermentation (Mercado et al. 2007, Wang and Liu

702013). The diversity of *S. cerevisiae* strains present in fermentations has been shown
71to play an important role in the characteristics of the final product (Egli et al. 1998,
72Pérez-Cooello et al 1999). Numerous molecular methods have been developed to
73study the ecology and population dynamics of *S. cerevisiae* strains (reviewed in
74Querol and Ramon 1996, Fernández Espinar et al.2006). Interdelta sequencing typing
75uses the variation of the number and position of the delta element, a repeated
76sequence that flanks the Ty1/Ty2 retrotransposon (Ness et al. 1993), that allows
77interpreting strain similarities and evolutionary or adaptive distance (Legras and Kerst
782003, Liu et al. 2014).

79 A succession of different *S. cerevisiae* strains are established during native as well
80as inoculated fermentations that could have positive or negative effects on the course
81of fermentation and wine quality (Mercado et al 2007,Wang and Liu 2013). Vezinhet
82et al. (1992) analyzed the evolution of *S. cerevisiae* strains isolated from spontaneous
83fermentations during six consecutive years. These authors concluded that the wide
84distribution of some strains in the studied areas and their presence over years,
85constitute evidence for the occurrence of specific indigenous strains representative of
86an enological region.

87 China is an important wine-producing country and while some studies have
88investigated indigenous yeast species and population dynamics during wine
89fermentation within local viticulture regions (Sun et al. 2009, Li et al. 2010, Li et al.
902011, Wang and Liu 2013, Sun et al. 2014); few studies (Wang and Liu 2013, Sun et
91al. 2014) have focused on the breadth of the diversity of *S. cerevisiae* wine genetic

resources of China. A study of human- and non-human-associated strains of China found novel distinct lineages only distantly related to the wine strain lineages (Wang et al. 2012). The genetic diversity and relatedness of indigenous wine *S. cerevisiae* resources have not been extensively compared with that of wine strains isolated from other geographic regions. Ningxia and Xinjiang provinces, where the strains in this study were isolated, are two of the oldest wine producing regions in China. Shanshan, Xinjiang in northwestern China belongs to a temperate continental climate, with an average temperature of 12°C. It is situated 92°22'E, 42°87'N with an average altitude of 3986 m. Qing Tongxia, Ningxia in north central China also belongs to a temperate zone (average temperature is 9°C) with an arid and semi-arid climate. It is situated 105°21' to 105°21' E, 37°36' to 38°15'N with an average altitude of 1118 m. A comparison of the genetic diversity of *S. cerevisiae* resources in different viticulture regions of China with isolates from other diverse geographical regions is of importance to the study of global *S. cerevisiae* ecology.

106 In the present study, interdelta sequence typing with improved primers (Legras
107and Karst 2003) was used as genetic marker for the distinction of *S. cerevisiae* strains.
108Dendrograms were constructed based on similarity among different patterns of bands
109and the genetic relationships of all strains were evaluated. The strains used in the
110study were either isolated from fermentations of different grape varieties in the
111Ningxia and Xinjiang Provinces in China or obtained from the Department of
112Viticulture and Enology Culture Collection at the University of California, Davis. The
113aims of the present work were to evaluate the genetic diversity and relatedness among

114 *Saccharomyces* strains of different geographic origin, to establish a strain collection to
115 preserve the *S. cerevisiae* genetic resources of China, and to identify strains useful for
116 further development for commercial wine production in China.

117 **Materials and Methods**

118 **Yeast strains.** Fifty-four isolates collected from fifteen spontaneous
119 fermentations of grapes grown in China and one commonly used commercial yeast,
120 Lavin RC212, were used in this study and obtained from the collections of the
121 College of Enology, Northwest A&F University, Yangling, Shaanxi, China. This set of
122 strains was selected on the basis of interdelta sequence profiles from a total of 349
123 isolates collected from fifteen spontaneous fermentations of grapes grown in
124 Shanshan, Xinjiang and Qing Tongxia, Ningxia. Fifty-nine yeast colonies were
125 isolated from six spontaneous fermentations of different commonly used grape
126 varieties: Red Globe, Small-berry Thompson Seedless, Big-berry Thompson Seedless,
127 Merlot, Mixed red (Cabernet Gernischt, Cabernet Franc, and Cabernet Sauvignon)
128 and Mixed white (Small-berry Thompson Seedless, and Big-berry Thompson
129 Seedless) in Xinjiang. Two hundred ninety isolates were obtained from nine
130 spontaneous fermentations of the grape varieties Cabernet Gernischt, Cabernet
131 Sauvignon, Cinsault, Merlot, Pinot Noir, Riesling, Sauvignon Blanc, Semillon, and
132 Yan73 in Ningxia (Pei 2009). The grape must fermentations were allowed to proceed
133 spontaneously at 25~28°C for 7~11 days until dry. Fermentations were sampled at
134 early, mid and the final stage of fermentation, and serial ten-fold dilutions were
135 inoculated onto WLN (Pallmann et al. 2001) and incubated for five days at 28°C.

136 These yeasts were differentiated and classified according to colony morphology and
137 color. *S. cerevisiae* isolates were purified and then maintained in 20% (v/v) glycerol at
138 -80 °C until further analysis, resulting in the selected set of 349 isolates for the
139 interdelta sequence analysis. The composition of the different grape musts is reported
140 in Supplemental Table 1 for Ningxia and in Supplemental Table 2 for the
141 fermentations from Xinjiang.

142 The fifty-four yeast strains selected from this larger population of isolates
143 represented the major strain clusters of interdelta sequence profiles identified in the
144 earlier preliminary study. The origins of the 54 isolates used in this study are shown in
145 Table 1. Identification of *S. cerevisiae* was confirmed by PCR-RFLP of the 5.8S-ITS
146 rDNA using restriction enzymes *Hae*III, *Hpa*II, and *Scr*FI as described by Li et al.
147 (2012). Strains were maintained in frozen stocks (glycerol, 20% v/v) at -80°C before
148 use. Note that a similar strain numbering system was independently used by Wang et
149 al. (2012) in their study but the strains are unrelated. We retained our numbering
150 system since that is the designation given to the strains in the Northwest A&F
151 University strain collection.

152 Other strains were obtained from the Wine Yeast and Bacteria Collection of the
153 Department of Viticulture and Enology at the University of California, Davis. The
154 data from all fifty-two *Saccharomyces* isolates listed in Table 1 of Liu et al. (2014)
155 were included in this study as the method of interdelta sequence analysis was
156 identical. These yeast strains were collected from California, France, Italy, Northern
157 Europe, and Spain (Liu et al. 2014).

158 **DNA extraction.** DNA from *S. cerevisiae* in the collection at the Northwest A&F

159University was extracted as described by Zhou et al. (2004).

160 **Interdelta sequence typing.** PCR amplification of the interdelta sequence was
161carried out using primers δ 12 (5'-TCAACAATGGAATCCCAAC-3') and δ 21 (5'-
162CATCTTAACACCGTATATGA-3') (Legras and Karst 2003). Amplification reactions
163and the DNA fragment separations were performed according to Liu et al. (2014). In
164brief, PCR amplifications were performed in a 25 μ L reaction volume containing 2.5
165 μ L of 10 $\times$ PCR buffer, 2.5 μ L 25 mM MgCL₂, 2.0 μ L 2.5 mM dNTPs, 1.25 μ L of 10
166 μ mole/L of each primer, 0.4 μ L of 5 U Taq DNA polymerase, 30-100 μ L of template
167DNA (amount varied dependent upon DNA quantification value of the sample) and
168double distilled H₂O to make the volume up to 25 μ L. The same volume of DNA
169sample (1 μ L) was added to each lane. PCR and interdelta typing analysis was
170performed multiple times (3 to 6 replicates) for each isolate in order to obtain a
171replicated stable interdelta pattern prior to analysis. Band positions were determined
172by reference to a standard DNA Ladder (100 bp Plus DNA Ladder (Fermentas)) to
173enable comparisons across gels. PCR reactions were repeated in cases where a low
174DNA content was evident on the gel.

175 **Cluster analysis of the strains.** The interdelta sequence patterns obtained after
176gel electrophoresis were used for the construction of a presence/absence matrix,
177taking into account the total number of different bands observed. The interdelta
178sequence patterns were obtained following electrophoresis. All visible bands were
179assigned a number based upon relative position to the DNA ladder. Each position was
180then assigned a "0" or a "1" to indicate absence or presence of the band, respectively.

181The 0/1 matrix was then used to generate the dendrograms. Similarities based on the
182Dice coefficient were calculated and UPGMA clustering was obtained using NTSYS
183software (Mercado et al. 2010).

184 The comparative cluster analysis of different strains integrates banding pattern
185data from two separate experiments. The data were combined in a single matrix.
186Although banding patterns differed there were some band positions in common in
187some strains across the two studies. The reagents used in the two studies were
188identical; however the PCR thermal cycler was different, a GeneAmp PCR System
1892700 was used in this study.

190 **Results**

191 **Interdelta sequencing typing of *S. cerevisiae* isolates in Xinjiang and Ningxia,**
192**China.** In a previous study 349 strains isolated from different native fermentations in
193China were screened by interdelta sequence typing. The number of strains tested in
194this analysis did not allow definitive comparisons of highly similar strains as each
195strain was only run once. However based on this preliminary analysis 54 different
196banding patterns were identified. These 54 strains were studied in more detail under
197conditions enabling conclusive comparisons of DNA band profile. Samples of the
198strains were run on gels multiple times and band positions calculated in comparison to
199the DNA ladder on each gel. Strains were run on different gels and yielded identical
200patterns on the replicate gels. This method generated stable replicate banding patterns
201for each isolate. The replicated interdelta sequence typing methodology enabled clear
202differentiation of forty-two distinct *S. cerevisiae* genotypes among the 54 *S.*

203 *cerevisiae* isolates of the first study. Twenty-one strains of *S. cerevisiae* isolated from
204 Xinjiang revealed eighteen different patterns (Figure 1). The analysis of thirty-three *S.*
205 *cerevisiae* isolates from Ningxia revealed the existence of twenty-four distinct profiles
206 (Figure 2) in other words, 24 differentiated strains. The results also showed that the
207 interdelta profile of genotype NX10 was identical to that of commercial yeast Lalvin
208 RC212, which has been used in this region as a commercial starter strain. In general,
209 the Xinjiang and Ningxia regions evaluated in this study exhibited different *S.*
210 *cerevisiae* populations. There were no identical strains between the regions. In
211 addition, comparison with the forty-four interdelta genetic patterns found among the
212 fifty-two *Saccharomyces* strains from Liu et al. (2014) revealed that no identical
213 strains were present between the studies.

214 **Genetic relationships among the strains from Xinjiang and Ningxia.** The PCR
215 amplification profiles obtained by interdelta sequence typing were used to obtain
216 independent presence-absence matrices for *S. cerevisiae* isolates from Xinjiang and
217 Ningxia, China. The dendrograms deduced by interdelta sequence typing are
218 presented in Figures 3 and 4, respectively. The dendrograms demonstrated that native
219 wine fermentations were conducted by a mixture of *S. cerevisiae* strains. In general a
220 minimum of two interdelta sequence profiles could be detected during each of the
221 spontaneous fermentations with the exception of the fermentation of Big-berry
222 Thompson Seedless. Some strains showed highly similar but not identical banding
223 patterns and are thought to represent genetically related strains. Six clusters of two
224 isolates each, cluster XJ4 and XJ5, XJ12 and XJ15, XJ2 and XJ7 (Figure 3); NX2 and

225NX3, NX29 and NX33, and NX9 and NX32 (Figure 4) showed highly conserved
226interdelta sequence patterns indicating that they are likely genetically distinct
227derivatives of the same strain. Two clusters of four isolates, cluster NX1, NX4, NX5,
228and NX8; cluster NX17, NX30, NX31, and NX26 also displayed conserved interdelta
229sequencing patterns (Figure 4) again suggesting a genetic relationship among the sets
230of strains. The *S. cerevisiae* isolates from Xinjiang that clustered together were from
231the same grape variety, but, in contrast, the isolates from Ningxia that clustered
232together were from more than two different grape varieties. NX10 from a native
233Riesling fermentation was indistinguishable from the commercial strain RC212.

234 A difference was observed in the genetic relationships of *S. cerevisiae* among
235strains isolated from table and wine grape varieties planted in Xinjiang (Figure 3).
236Both table and wine grapes are used for wine production in Xinjiang. Four groups
237containing nine, eight, three, and one isolate, respectively, were distinguished with a
238Dice coefficient of 0.448. All nine isolates in group I and all the 3 isolates in group III
239were obtained from the table grape varieties Red Globe, Small-berry Thompson
240Seedless, Big-berry Thompson Seedless, and Mixed white (Small-berry Thompson
241Seedless and Big-berry Thompson Seedless). Group II had seven isolates from wine
242grape varieties of Merlot and Mixed red (Cabernet Gernischt, Cabernet Franc, and
243Cabernet Sauvignon), and one isolate from the table grape variety Red Globe. Group
244IV only included one genotype, XJ3, also from Red Globe (Figure 3). Thus the
245majority of isolates from the four different table grape fermentations were genetically
246similar in banding pattern and clustered together. In addition, a difference was

247observed in the genetic relationships of *S. cerevisiae* among strains isolated from red
248and white wine grape varieties planted in Ningxia (Figure 4). Five groups are
249indicated in Figure 4, when the Dice coefficient is 0.674. Groups I, II, and III
250consisted of isolates from more than three grapes varieties, while group V only
251included isolates from Pinot Noir. Group IV included only 1 isolate, NX20, from
252Cabernet Sauvignon. Most of the *S. cerevisiae* isolates included in group I were
253isolated from red grape varieties (8 of the 13 isolates in group I), while isolates in
254group III were mainly from white grape varieties (9 of the 12 isolates in group III).
255All the isolates in group II were from the red grape varieties of Cabernet Sauvignon,
256Merlot, and Cinsault (Figure 4).

257 **Genetic relationships among the strains from different countries.** Genetic
258relatedness was also evaluated by constructing a dendrogram compiled from all
259interdelta sequence patterns in this study and Liu et al. (2014) (Figure 5). Differences
260were observed in the genetic relationships between the Chinese strains and the strains
261from the UC Davis collection. The similarity of strains was correlated with their
262geographical regions of origin: Ningxia strains were closer to the Xinjiang strains,
263while a clear separation between the indigenous Chinese and the UC Davis strains
264was observed. The dendrogram produced by interdelta sequence typing revealed five
265groups containing fifty-one, four, forty-eight, three, and one isolate each when the
266Dice coefficient is 0.418 (Figure 5). The largest group, group I, contained fifty
267isolates from China and one from California (UCD2211, *S. servazzii*). There is a
268difference observed in the genetic relatedness between Xinjiang and Ningxia

269indigenous *S. cerevisiae* strains. Group I could be further divided into five subgroups,
270I-1 to I-5, when the Dice coefficient is 0.506. Most of the *S. cerevisiae* isolates
271included in subgroups I-1 (5 of 6) and I-5 (6 of 7) as well as all three in I-4 were
272isolated from Xinjiang, while isolates in I-3 (29 of 31) and I-2 (3 of 3) were mainly
273from Ningxia (Figure 3). Group II displayed a high similarity with XJ19 (Xinjiang),
274UCD587 (a winery isolate), UCD2515 and UCD2516 (both are lab strains known to
275be related to each other). Forty-eight out of fifty-two *Saccharomyces* isolates from the
276UC Davis collection were clustered in group III. XJ2, XJ7, and XJ20 were clustered
277in group IV. Group V consisted only of XJ3. Clusters IV and V containing XJ2, XJ7,
278XJ20, and XJ3 had a low degree of similarity with other Chinese indigenous
279genotypes and UC Davis collection strains (Figure 5). In addition, this dendrogram
280showed that identical isolates were found only in the same geographic regions with
281the exception of UCD522 (commercial yeast) and UCD514 (origin in Spain).

282 **Discussion**

283 Understanding the genetic diversity of *S. cerevisiae* strains from different
284geographical origins can make an important contribution towards delineating the
285genetic distance of these strains as well as providing genetic material for further strain
286development. The genetic diversity of indigenous *S. cerevisiae* was investigated
287during the spontaneous fermentations of grape must in Xinjiang and Ningxia, China.
288Eighteen distinct interdelta profiles were found in Xinjiang, and twenty-four in
289Ningxia. Forty-two different *S. cerevisiae* strains were distinguished out of a total of
290three hundred and forty nine isolates analyzed. All forty-two of these isolates were

291unique when compared to a set of strains from other major international wine
292producing regions (Dendrogram 3 groups I, IV, V).

293 Lavin RC212, showing the same interdelta sequence patterns as genotype NX10
294isolated from Ningxia was detected during the spontaneous fermentations in this
295study. Similar to other studies, commercial yeasts were detected in fermentations
296without inoculation (Santamaria et al. 2005, Mercado et al. 2007, Sun et al. 2014). In
297this study, the detection of Lavin RC212 colonizing a spontaneous fermentation could
298be explained by the winery practice of dumping grape skins on the road for drying.
299Valero et al. (2005) analyzed the dissemination and survival of commercial wine yeast
300in the vineyards near wineries and they suggested that the dispersion of commercial
301strains is mainly mediated by water run-off and derived from macerated grape skin at
302dumping sites. Drying the grape skins on the roads for further processing is a normal
303practice at the Imperial Horse Winery, Qing Tongxia, Ningxia, China, where the
304spontaneous fermentations were conducted. It is understandable that this practice
305could have contributed to the dissemination and survival of Lavin RC212 in the
306vineyards and wineries, and its occurrence in spontaneous fermentations.

307 In this study, comparison between eighteen and twenty-four different *S.*
308*cerevisiae* patterns by interdelta sequence typing revealed that yeast strains from
309Xinjiang and Ningxia did not share the same interdelta profiles. The same
310observations made in the Western Cape, South Africa (Khan et al. 2000) showed that
311different *S. cerevisiae* strains were present at different regions in the different climate
312zones. In addition, the differences could be attributed to the fact that the grape

313 varieties studied were different in these two regions: table and wine grape varieties in
314 Xinjiang vs. wine grape varieties in Ningxia. This result agrees with a previous study
315 that demonstrated the impact of grape variety on yeast diversity (Mercado et al. 2011).
316 Further, this study suggests that these two winemaking regions are biologically
317 isolated from each other.

318 Geographic location and ecological niches are both thought to play a significant
319 role in *Saccharomyces* strain diversity (Bisson 2012). In comparison with strains
320 isolated from other winemaking regions, the Ningxia and Xinjiang strains showed a
321 high degree of similarity. This suggests that the indigenous Chinese strains are distinct
322 from European and new world lineages. Nearly identical strains were only found in
323 the wine samples collected in the same viticulture region with the exception of
324 UCD522 (commercial yeast) and UCD514 (origin in Spain). These results are in
325 agreement with previous studies on geographically close regions (Versavaud et al.
326 1995) and widely distant geographic regions (Goddard et al. 2010). According to
327 Ezeronye and Legras (2009), who studied the genetics of *S. cerevisiae* strains isolated
328 from palm wine in eastern Nigeria, geographic and/or ecological isolation results in a
329 specific population of *S. cerevisiae*. These analyses have led to the conclusion that
330 geographic location plays a significant role in genetic divergence. Strain XJ19
331 displayed a high similarity with UCD587 (a California must isolate), UCD2515
332 (YPH500) and UCD2516 (BY4743) (two genetically related lab strains with
333 California origins (Mortimer and Johnston 1996). The relatedness of these four strains
334 may imply a common origin or a commonality of evolutionary forces in the wild.

335 Interestingly as a group the Chinese wine strains appear to show greater overall
336diversity as compared to the wine isolates from the rest of the world. This is
337consistent with the narrowness of the Wine European lineage previously described
338(Wang et al. 2012) as well as the observations of overall greater diversity of natural *S.*
339*cerevisiae* isolates from China as compared to other regions (Liti 2015). The greater
340natural diversity of isolates of *S. cerevisiae* from China many represent an untapped
341genetic reservoir for strain improvement and breeding programs. Knowledge about
342indigenous yeast strains can also help preserve and employ the most representative
343strains from a wine region (Tristezza et al. 2014).

344 **Conclusion**

345 This study investigated the genetic diversity of *S. cerevisiae* in Ningxia and
346Xinjiang, China, which has not previously been examined. The results of this study
347showed that different *S. cerevisiae* strains were associated with different viticulture
348regions in China. In addition, the results demonstrated that a commercial yeast was
349detected in spontaneous fermentations at one winery. Ningxia and Xinjiang are two of
350the best known viticulture regions in China; therefore, the preservation of biodiversity
351and genetic resources of indigenous yeasts is very important in these regions.

352

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357

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Figure Legends

458

459

460**Figure 1:** Interdelta sequence profiles of the 21 genetically distinct *S. cerevisiae*
461isolates in Xinjiang, China. M: 100bp Plus DNA Ladder; 1: XJ1, 2: XJ2, 3: XJ3, 4:
462XJ4, 5: XJ5, 6: XJ6, 7: XJ7, 8: XJ8, 9: XJ9, 10: XJ10, 11: XJ11, 12: XJ12, 13: XJ13,
46314: XJ14, 15: XJ15, 16: XJ16, 17: XJ17, 18: XJ18, 19: XJ19, 20: XJ20, 21: XJ21.

464

465**Figure 2:** Interdelta sequence profiles of the 33 genetically distinct *S. cerevisiae*
466isolates in Ningxia, China. M: 100bp Plus DNA Ladder; 1: NX1, 2: NX2, 3: NX3, 4:
467NX4, 5: NX5, 6: NX6, 7: NX7, 8: NX8, 9: NX9, 10: NX10, 11: NX11, 12: NX12, 13:
468NX13, 14: NX14, 15: NX15, 16: NX16, 17: NX17, 18: NX18, 19: NX19, 20: NX20,
46921: NX21, 22: NX22, 23: RC212, Commercial *S. cerevisiae* strain RC212, 24: NX23,
470i25: NX24, 26: NX25, 27: NX26, 28: NX27, 29: NX28, 30: NX29, 31: NX30, 32:
471NX31, 33: NX32, 34: NX33.

472

473**Figure 3:** Unweighted Pair Group Method with Arithmetic mean (UPGMA)
474dendrogram showing genetic relatedness of the *S. cerevisiae* isolates obtained from
475Xinjiang, China. Four distinct groupings of strains were evident with a Dice
476coefficient of 0.448. The black line represents the Dice coefficient and groups are
477designated by use of Roman numerals.

478

479**Figure 4:** UPGMA dendrogram showing genetic relatedness of the *S. cerevisiae*
480isolates obtained from Ningxia, China. Five distinct groupings of strains were evident

481with a Dice coefficient of 0.674. The black line represents the Dice coefficient and
482groups are designated by use of Roman numerals.

483

484**Figure 5:** UPGMA dendrogram generated by cluster analysis of interdelta region
485profiles obtained of *Saccharomyces* from our culture collection and Department of
486Viticulture and Enology Culture Collection in University of California, Davis. Five
487distinct groupings of strains were evident with a Dice coefficient of 0.418. Two of
488these groups were large: group I (51 isolates) and group III (48 isolates). Using a
489higher Dice coefficient (0.506), the two larger groups can be differentiated in to
490subgroups. The black line represents the Dice coefficient and groups are designated
491by use of Roman numerals. The subgroups are designated by use of Arabic numerals.