Short Communication

Genetic identification of cultured *Saccharomyces* yeasts from Asia

Gennadi I. Naumov,* Elena S. Naumova, and Irina V. Korshunova

State Institute for Genetics and Selection of Industrial Microorganisms,
I-Dorozhnyi 1, Moscow 113545, Russia

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In Europe, cultured *Saccharomyces* yeasts are represented by two biological species, *S. cerevisiae* and *S. bayanus*, and one hybrid taxon *S. pastorianus* (syn. *S. carlsbergensis*), as proved by genetical and molecular analyses (Naumov, 1987; Naumov et al., 1983, 2000b; Vaughan Martini and Kurtzman, 1985; Vaughan Martini and Martini, 1987). Cultured *Saccharomyces* strains from Japan are known to belong to the *S. cerevisiae* species, as well (Yamada et al., 1993). Rare wild isolates of *S. cerevisiae* and *S. bayanus* were isolated in Europe and North America (Naumov et al., 1992a, 1996, 1998). Besides, wild *S. cerevisiae* strains were found in Asia (Naumov and Naumova, 1991; Naumov and Nikonenko, 1988). Correct identification of cultured and wild *Saccharomyces* yeasts promotes use of their natural gene pool in basic and applied investigations (Naumov, 1996). In spite of the fact that the genome of some laboratory lines of *S. cerevisiae* has been sequenced in the frame of the international project, it is practically not studied at the population level. The known gene sequences compose only the genetic skeleton of the species while its body, the real genomes of natural populations, has yet to be investigated.

The aim of the present work was to identify the indigenous *Saccharomyces* yeasts isolated from Asian

beverage and food fermentations. The *Saccharomyces* strains used are listed in Table 1. As genetic references for the biological species *S. cerevisiae*, we used highly fertile monosporic cultures of homothallic strain VKM Y-502 (Naumov, 1987) and haploid strain X2180-1A (Yeast Genetic Stock Center, University of California), marked by auxotrophic *ade1* and *ade2* mutations (red colonies), respectively. The latter tester also carried natural *gal2* marker (non-fermentation of galactose). The methods for cultivation and hybridization of yeasts have been described elsewhere (Naumov et al., 1986). Hybrids of homothallic strains were obtained by the “spore-to-spore” method using a micromanipulator. Hybrids of heterothallic yeasts were obtained by mass mating of haploid cells and by isolation of zygotes with a micromanipulator. The hybrid nature of cultures was determined by analyzing the meiotic segregation of the control markers.

Only fertile strains are suitable for genetic hybridization analysis. First of all, the sporulating strains were selected (Table 1). Spore viability and the type of life cycle (homo- or heterothallism) were determined. Five to twelve tetrads from each strain were dissected. Most strains showed high ascospore viability (83–100%). Strain S8BM-32 had ascospore viability of 57%. Four strains, NCYC 2402, ATCC 52922, ATCC 38618, and IFO 0289, showed low fertility (20–25%). Monosporic cloning over one or several generations was used in order to obtain fertile clones. For example, ascospore viability of strain ATCC 52922 was increased up to 83% only in the fourth generation. Most

* Address reprint requests to: Dr. Gennadi I. Naumov, State Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 113545, Russia.
E-mail: gnaumov@yahoo.com

Table 1. Strains of *Saccharomyces cerevisiae* identified by genetic analysis.

Strain designation ^a	Source of isolation	Reference or author
NCYC 2402	Phloem sap of coconut, Sri Lanka	S. Chandrani Wijeyaratne
S11F-3	Phloem sap of <i>Caryota urens</i> , Sri Lanka	S. Chandrani Wijeyaratne
S8BM-32	Phloem sap of <i>Caryota urens</i> , Sri Lanka	S. Chandrani Wijeyaratne
S8BM-30	Phloem sap of <i>Caryota urens</i> , Sri Lanka	S. Chandrani Wijeyaratne
ATCC 66349	Candied apple, Japan	Tokuoka et al., 1985
ATCC 66348	Soil, Japan	Tokuoka et al., 1985
ATCC 52922	Tapuy, Philippines	H. Sakai
ATCC 38618	Concentrated orange juice	B. J. Juven
JCM 2985	Fermented fish, Thailand	Suzuki et al., 1987
JCM 3529	Fermented shrimp, Thailand	Suzuki et al., 1987
CBS 1576	Sap of <i>Arenga</i> palm, Sulawesi, Indonesia	A. Reyne
IFO 0289	Moromi of Chinese wine kaoliangchiu	Yamada et al., 1993

^aThe acronyms for culture collections are: ATCC=American Type Culture Collection, Manassas, VA; CBS=Centraalbureau voor Schimmelcultures, Utrecht, Holland; JCM=Japan Collection of Microorganisms, RIKEN, Saitama, Japan; IFO=Institute for Fermentation, Osaka, Japan; NCYC=National Collection of Yeast Cultures, Norwich, UK; VKM=All-Russian Collection of Microorganisms, Moscow, Russia. The strains received from S. Chandrani Wijeyaratne are maintained at the yeast collection of Dept. of Botany, University of Sri Jayawardenepura, Nugegoda, Sri Lanka.

strains were homothallic. Three strains, NCYC 2402, S8BM-30, and S8BM-32, showed a delayed self-diploidization; their monosporic cultures consisted, for the most part, of haploid cells having one mating type and diploid sporulating cells. Strain S11F-3 was heterozygotic on the homothallism gene (probably *HO*), as judged from the monogenic segregation on homo-heterothallism (not shown).

Currently, six sibling species are known in the *Saccharomyces sensu stricto* complex (Naumov et al., 2000a). The species display similar basic karyotypic characters, i.e. the same haploid number of chromosomes ($n=16$) and the same range of chromosomal bands (from 250 to 2,200 kb). *S. bayanus* and *S. cariocanus* have species-specific karyotypes, while *S. cerevisiae*, *S. paradoxus*, *S. kudriavzevii*, and *S. mikatae* display similar banding profiles. Monosporic cultures of the strains under examination were used for electrophoretic karyotyping. Cloning of the strains from one spore led to the homozygosity of chromosomal sets and to an elimination of extra chromosomes. *S. cerevisiae* standard strain YNN 295 with known sizes and order for its chromosomes was used for comparison (Fig. 1, lane 1). The karyotype patterns of the strains studied were similar to that of the standard strain (Fig. 1, lanes 2–12). This indicates that the strains cannot be assigned either to *S. bayanus* or *S. cariocanus*. Among the yeasts studied, chromosome length poly-

Fig. 1. Molecular karyotyping of indigenous Asian *Saccharomyces cerevisiae* strains.

The chromosomal DNAs were separated by CHEF electrophoresis (Naumov et al., 1992a) at 200 V for 15 h with a switching time of 60 s and then for 9 h with a switching time of 90 s. The running buffer was 0.5×TBE cooled to 14°C. The chromosome sizes refer to the chromosomes of the standard strain YNN 295 (Bio-Rad, CA, USA). All cultures studied are monosporic. 1, YNN 295; 2, NCYC 2402; 3, S11F-3; 4, S8BM-30; 5, S8BM-32; 6, ATCC 66348; 7, ATCC 52922; 8, ATCC 38618; 9, JCM 2985; 10, JCM 3529; 11, CBS 1576; 12, IFO 0289.

morphism was evident for small, large and middle-sized chromosomes. Three separate chromosomes are seen in most of the strains. In several strains,

Table 2. Genetic analysis of hybrids of Asian *Saccharomyces* strains (monosporic cultures) with reference testers of the biological species *S. cerevisiae* (VKM Y-502 and X2180-1A).

Origin of hybrids ^a	Number of spore pairs crossed	Number of zygotes obtained	Number of tetrad isolated	Proportion of viable ascospores (%)	Segregation of control marker ^b 2ADE : 2ade
NCYC 2402×502	34	2	41	65	13
S11F-3×502	37	4	20	71	9
S8BM-32×X2180-1A	—	—	14	86	8
S8BM-30×502	33	4	27	63	11
ATCC 66349×X2180-1A	—	—	14	86	7
ATCC 66348×502	32	1	20	85	12
ATCC 52922×502	37	2	41	72	15
ATCC 38618×502	36	5	41	73	14
JCM 2985×502	31	4	13	77	3
JCM 3529×502	31	3	13	88	8
IFO 0289×X2180-1A	—	—	35	86	24
CBS 1576×502	38	5	30	87	19

^aEach hybrid analyzed originated from one zygote isolated by the micromanipulator. Hybrids of strains S8BM-32, ATCC 66349, and IFO 0289 were obtained by mass crossing of haploid cells.

^bNumber of tetrads analyzed. For hybrid of strain IFO 0289, segregation on the *gal2* gene is given. The ability of segregants to ferment galactose was determined on pH-indicator medium.

bands with stronger relative intensity were observed, suggesting that these bands corresponded to doublets. In three strains, S11F-3, S8BM-32, and ATCC 66348 (Fig. 1, lanes 3, 5, and 6, respectively), chromosomes VI and III comigrated. In strain ATCC 38618 (Fig. 1, lane 8) chromosomes I and VI migrated as a doublet. Six strains, NCYC 2403, S11F-3, S8BM-30, ATCC 52822, CBS 1576, and IFO 0289 (Fig. 1, lanes 2–4, 7, 11, and 12), displayed chromosome XI of larger size than in the other strains. Also, chromosomes II and XIV probably comigrate in several strains. Unlike the tester strain YNN 295, in all strains chromosomes XIII and XVI migrated in doublets. To summarize, the *Saccharomyces* strains under examination each showed a characteristic chromosomal pattern, suggesting their belonging to *S. cerevisiae*. It is well known that karyotype patterns of cultured *S. cerevisiae* strains are characterized by pronounced chromosome length polymorphisms allowing molecular typing of individual strains (Naumov et al., 1992b; Vezinhet et al., 1990; Yamamoto et al., 1991). In contrast, the polymorphism of karyotypes is very low among *S. paradoxus* isolates and *S. cerevisiae* strains isolated from nature (Naumov et al., 1992b, 1996, 1998).

Fine taxonomic status of twelve Asian cultured

strains was determined by analyzing hybrids of their fertile monosporic cultures and genetic testers of the biological species *S. cerevisiae* (Table 2). In all cases spores copulated with normal frequency. The hybrids obtained were rather fertile (65–93% ascospore viability) and showed normal monogenic segregation of the control markers. When tetrads formed rarely, triad and random spore analyses also revealed monogenic segregation of the control markers (not shown). Thus, the genetic data obtained strongly indicated that all 12 strains studied belonged to the biological species *S. cerevisiae*.

None of the four wild species (*S. paradoxus*, *S. kudriavzevii*, *S. mikatae*, and *S. cariocanus*) was found among the *Saccharomyces* yeasts isolated from indigenous foods in Asia. In the conditions of fermentation processes, wild species probably cannot compete with the strong fermenting cultured yeast *S. cerevisiae*, which is able to produce a large amount of ethanol. The absence of the *S. bayanus* species in the fermentation processes of the countries with hot climate is probably connected with its strong cryotolerance, determining the distribution of this yeast (Naumov et al., 2000b).

The genome of the *S. cerevisiae* species at the mo-

lecular and chromosomal levels is diverged significantly from its five sibling species (Fischer et al., 2000). It would be worthwhile to study the diversity of the *S. cerevisiae* genome using cultured strains isolated from different fermentations all over the world. The fertile lines of Asian cultured *S. cerevisiae* yeasts obtained at the present study can be used for such kind of investigations. On the other hand, wild strains of *Saccharomyces sensu stricto* complex are of interest for investigating new biological species and varieties, i.e. for studying the macroevolution process of these yeasts.

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