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
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 \textit{HO}), as judged from the monogenic segregation on homothallism (not shown).

Currently, six sibling species are known in the \textit{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). \textit{S. bayanus} and \textit{S. cariocanus} have species-specific karyotypes, while \textit{S. cerevisiae}, \textit{S. paradoxus}, \textit{S. kudriavzevii}, and \textit{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. \textit{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 \textit{S. bayanus} or \textit{S. cariocanus}. Among the yeasts studied, chromosome length polymorphism was evident for small, large and middle-sized chromosomes. Three separate chromosomes are seen in most of the strains. In several strains,
bands with stronger relative intensity were observed, suggesting that these bands corresponded to dou-
blets. In three strains, S11F-3, S8BM-32, and ATCC 66348 (Fig. 1, lanes 3, 5, and 6, respectively), chromo-
somes 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, chromo-
somes 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 exami-
nation each showed a characteristic chromosomal pat-
tern, suggesting their belonging to S. cerevisiae.

It is well known that karyotype patterns of cultured S. cerevisiae strains are characterized by pronounced chro-
mosome length polymorphisms allowing molecular typ-
ing of individual strains (Naumov et al., 1992b; Vezin-
het 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 seg-
regation 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. ku-
driavzevii, S. mikatae, and S. cariocanus) was found
among the Saccharomyces yeasts isolated from in-
digenous foods in Asia. In the conditions of fermenta-
tion 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 fermenta-
tion processes of the countries with hot climate is
probably connected with its strong cryotolerance, de-
termining the distribution of this yeast (Naumov et al.,
2000b).

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

<table>
<thead>
<tr>
<th>Origin of hybrids</th>
<th>Number of</th>
<th>Number of</th>
<th>Number of</th>
<th>Proportion of viable</th>
<th>Segregation of control marker</th>
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<tr>
<td></td>
<td>spore pairs</td>
<td>zygotes</td>
<td>tetrads</td>
<td>(% of viable</td>
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<tr>
<td></td>
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<td>obtained</td>
<td>isolated</td>
<td>ascospores)</td>
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<tr>
<td>NCYC 2402×502</td>
<td>34</td>
<td>2</td>
<td>41</td>
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<td>13</td>
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<tr>
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<td>37</td>
<td>4</td>
<td>20</td>
<td>71</td>
<td>9</td>
</tr>
<tr>
<td>S8BM-32×X2180-1A</td>
<td>—</td>
<td>—</td>
<td>14</td>
<td>86</td>
<td>8</td>
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<tr>
<td>S8BM-30×502</td>
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<td>4</td>
<td>27</td>
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<tr>
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<td>—</td>
<td>—</td>
<td>14</td>
<td>86</td>
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</tr>
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<td>38</td>
<td>5</td>
<td>30</td>
<td>87</td>
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</table>

*a Each 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.

*b Number 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.
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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