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EXPERIMENTAL ARTICLES

Genetic Identification of African Cultured Yeasts of the Genus *Saccharomyces*

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Abstract—Based on the results of genetic analysis and molecular karyotyping, a total of 11 strains involved in fermentation processes employed in various regions of Africa were identified as representatives of the species *Saccharomyces cerevisiae*. These stains exhibited a high degree of chromosomal polymorphism. The obtained fertile genetic lines of the studied African strains may be of considerable interest for evolutionary genetics and breeding of yeasts of the genus *Saccharomyces*.

Keywords: yeasts, *Saccharomyces cerevisiae*, genetic analysis, molecular karyotyping, Africa.

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In recent years, due to the growing number of genomic projects, a great deal of attention has been devoted to the study of the biological diversity of yeasts of the genus *Saccharomyces*. Our studies demonstrated that the genus *Saccharomyces* includes the following species: *S. cerevisiae* (T), *S. arboricola, S. bayanus, S. cariocanus, S. kudriavzevii, S. mikatae*, and *S. paradoxus* [1–5]. The geographic range of the *Saccharomyces* biological species is in Europe, Asia, and North America (*S. cerevisiae, S. bayanus*, and *S. paradoxus*) [6–27] and Europe and Asia (*S. kudriavzevii*) [3, 18, 28]; as endemic species, *S. mikatae* and *S. arboricolus* were found in Asia, while *S. cariocanus* was found in South America (Brazil) [3, 5]. A divergent population of *S. paradoxus* was found in Hawaii [29].

However, African *Saccharomyces* yeasts remain poorly studied. Molecular biological techniques were used for identification of a large collection of strains, tentatively identified as *S. cerevisiae* and isolated from sorghum beer (Ghana and Burkina Faso) and from fermented corn dough (Ghana) [30–32].

The goal of the present work was genetic identification of 11 cultured strains of *Saccharomyces* isolated in different regions of Africa.

MATERIALS AND METHODS

The studied isolates and reference strains of the genus *Saccharomyces* are listed in Table 1. Their genotypes were designated in accordance with the international system of yeast gene designations.

The yeast strains were grown on complete YPD medium containing the following (g/l): bacto agar (Difco, United States), 20; glucose (Reakhim, Russia), 20; yeast extract (Difco), 10; and peptone (Difco), 20. Spore formation was induced on the standard acetate medium containing the following (g/l): bacto agar, 20; CH_3COONa, 10.0; and KCl, 5.0. The tests for fermentation of specific sugars and the relevant media were previously described in [34]. All strains were incubated at 28°C. Spores were isolated using a Carl Zeiss (Jena, GDR) micromanipulator equipped with a glass needle after digestion of ascus wall with an enzymatic extract prepared from the stomach juice of the garden snail *Helix pomatia*. The hybrids of heterothallic strains were obtained by mass crossing of the cells of opposite mating types on complete medium and by subsequent isolation of the zygotes using the micromanipulator. The hybrids of homothallic strains obtained by the spore-to-spore method and the hybrids of homo- and heterothallic strains obtained by the spore-to-haploid cell method were also isolated using the micromanipulator [35].

Isolation of the chromosomal DNA was previously described in [14]. Electrophoresis of the chromosomal DNAs was carried out using the CHEF-DR II Electrophoresis System (Bio-Rad, United States) at 200 V for 24 h (for 15 h with a switching time of 60 s and for 9 h with a switching time of 90 s). The electrophoresis buffer (0.5 ± TBE), cooled to 14°C, contained the following: Tris–HCl, 45 mM; boric acid, 45 mM; EDTA, 10 mM; pH 8.2). After electrophoresis, the gels were stained with ethidium bromide, washed with distilled water, and photographed under UV light.

RESULTS AND DISCUSSION

Cloning of monosporous cultures. Only fertile homozygous strains are to be used for genetic analyses. Therefore, monosporous cultures of all the studied

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Table 1. *Saccharomyces* strains, monosporous cultures of which were used for genetic analysis

Strain*	Source	Monosporous culture genotype	Reference or author
MUCL 30909	Cassava, Burundi	*HO*	V. Roberts
Dji2	Palm wine, Djibouti	*MAT*a *SUC mal GAL mel*	M. Aigle
YO 495	Winery, Robertson, South Africa	*HO SUC MAL GAL MEL*	N. Jolly
YO 504	Winery, Riebeeck Kasteel, South Africa	*HO SUC MAL GAL MEL*	N. Jolly
YO 614	Same	*HO SUC MAL GAL MEL*	N. Jolly
No. 26-1-10	Corn dough, Ghana	*MAT*a *SUC MAL GAL MEL*	[32]
No. 34	Same	*MAT*a *SUC MAL GAL MEL*	[32]
No. 127	"	*MAT*a *SUC MAL GAL MEL*	[32]
No. 134	"	*MAT*a *SUC MAL GAL MEL*	[32]
CBS 403	Ginger Beer, West Africa	*HO mal*	A. Guillermond
CBS 405	Billi Wine, West Africa	*HO mal*	A. Guillermond
Reference *S. cerevisiae* strains			
S288C	Genetic line	*MAT*α *SUC2 mal gal2 mel*	R.K. Mortimer, [33]
X2180-1A	Same	*MAT*a *SUC2 mal gal2 mel*	R.K. Mortimer, [33]
YNN 295	"	*MAT*α *ura3 ade1 ade2 lys2*	Bio-Rad
VKM Y-502	Winery, Far East, Russia	*HO mal ad*	[1, 6]
M-437	Winery, Transcarpathia, Ukraine	*HO MAL*	[1, 6]

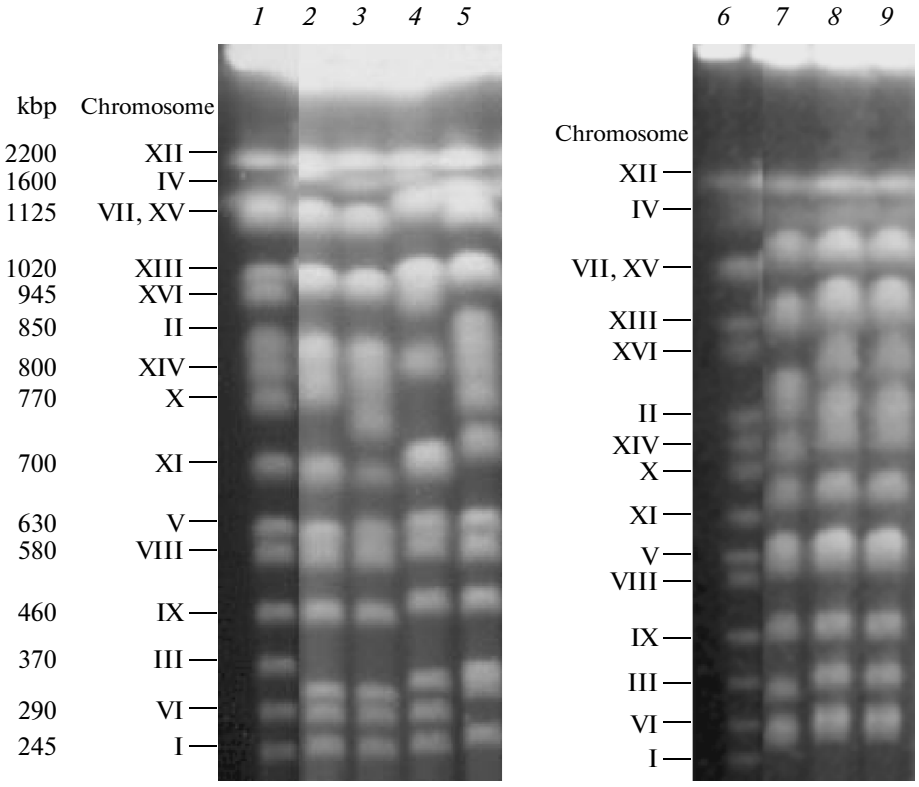
* Abbreviated collection names: MUCL = Mycothéque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; YO = ARC Infruitec-Nietvoorbij Yeast Genebank, Stellenbosch, South Africa; CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; VKM = All-Russian Collection of Microorganisms, Moscow, Russia; M = Magarach Collection of the National Institute for Vine and Wine, Yalta, Ukraine. For the heterothallic strains Dji2, nos. 26-1-10, 34, 127, and 134 the monosporous haploid cultures Dji2-2A, nos. 26-1-10:73, 34:2-4, 127:5-4, and 134:7-1, respectively, were used. The monosporous culture of strain VKM Y-502 was labeled by the UV-induced *ade* mutation (red colonies).

strains were obtained. Table 2 shows the spore viability and the types of life cycle (homo- and heterothallism) of yeasts. The monosporous cultures of heterothallic strains nos. 26-1-10, 34, 127, and 134 producing fertile hybrids with the test strains S288C and X2180-1 A were selected; the initial low-fertility strains were probably heterozygous with respect to the genetic factors controlling spore viability.

Karyotypic analysis. At present, members of the genus *Saccharomyces* can be easily diagnosed using their molecular karyotype. The species belonging to this genus have haploid genus-specific sets of chromosomes ($n = 16$); the sizes of chromosomes always range from 250 to 2200 kbp. In addition, the species *S. bayanus* and *S. cariocanus* have species-specific karyotypes, whereas *S. cerevisiae, S. paradoxus, S. kudriavzevii, S. mikatae*, and *S. arboricola* share similar karyotypes [2, 3, 5, 16]. Cloning from one spore results in the strain homozygosity, with respect to the sizes of homologous chromosomes as well; excessive chromosomes are eliminated. For comparison, we used the karyotypic standard, *S. cerevisiae* YNN 295 (Fig. 1, lanes *1* and *6*). The sizes of most of the chromosomes in the studied strains varied to some extent. Only the most significant differences will be considered in detail. The lack of some standard-sized chromosomes, as well as the high intensity of the neighboring chromosomes, indicate that, in the cells of strain no. 34, chromosomes XIV and II form a doublet (Fig. 1, lane *2*). At the same time, chromosomes XIII, XVI, and II of strain MUCL 30909 migrate as a triplet, whereas chromosomes X and XI migrate as a doublet (Fig. 1, lane *4*). Since the karyotype of strain no. 26-1-

Table 2. Ascospore viability and homo- and heterothallism of the African *Saccharomyces* strains

Strain	Number of obtained tetrads	Ascospore viability (%)	Hetero- or homothallism
MUCL 30909	7	93	Homo
YO 495	9	100	Homo
YO 504	10	100	Homo
YO 614	11	86	Homo
CBS 403	16	96	Homo
CBS 405	24	100	Homo
No. 26-1-10	28	25	Hetero
No. 34	36	35	Hetero
No. 127	6	63	Hetero
No. 134	34	46	Hetero



Molecular karyotypes of African yeasts of the genus *Saccharomyces*: karyotypic standard, *S. cerevisiae* YNN 295 (*1, 6*), no. 34 (*2*), no. 134 (*3*), MUCL 30909 (*4*), Dji2 (*5*), YO 495 (*7*), YO 504 (*8*), and YO 614 (*9*). Karyotypes of the monosporous cultures of the studied strains are given. The order and sizes of chromosomes (kbp) are given according to YNN 295.

10 was identical to the karyotype of strain no. 134, it is not listed here. The karyotypes of strains CBS 403 and CBS 405 have been published in [36]. While the results of the karyotypic analysis confirm classification of the studied strains as members of the genus *Saccharomyces*, they are insufficient to unambiguously determine their species affiliation.

Genetic analysis. Analysis of the fertility of yeast hybrids with the standard species tester strains is the only method confirming the unambiguous affiliation of yeasts to species of the genus *Saccharomyces*. Different species of the genus *Saccharomyces* are able to cross with each other due to the universality of their mating types; however, they produce sterile hybrids with unviable ascospores (products of meiotic division). Both homo- and heterothallic strains were able to cross with the test cultures of *S. cerevisiae* at a normal frequency (Table 3). Usually, all hybrids were fertile (with the ascospore viability of 65–98%); however, the hybrids of strain MUCL 30909, characterized by the predominance of viable dyads, were found to be less fertile (38%). It may be suggested that this is due to translocation, which, presumably, affects chromosome X. In strain MUCL 30909, unlike other strains, chromosome X migrates together with chromosome XI (see above). All hybrids showed monogenic segregation for one or two control markers. The results of our genetic analysis unambiguously characterize all the studied African strains as members of the species *S. cerevisiae*.

Wild *Saccharomyces* species obviously cannot compete with the strong fermentative *Saccharomyces* cultured strains, which, as is well known, are able to produce large amounts of ethyl alcohol. The fact that in hot regions *S. bayanus, S. kudriavzevii*, and *S. arboricola* are not involved in fermentation processes can also be attributed to their high cryotolerance, which probably determines the distribution of these yeasts.

Hence, we identified 11 *S. cerevisiae* strains isolated from the fermentation processes employed in various regions of Africa. The obtained fertile genetic lines are potentially a valuable genetic pool for yeast breeding. The genomes of the studied African strains are of considerable interest to scientists studying the origin of European and Asian yeast strains, as well as of their wild relatives on different continents.

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Table 3. Genetic analysis of the obtained hybrids of the African *Saccharomyces* strains (monosporous cultures) with *S. cerevisiae* tests strains (S288C, X2180-1A, VKM Y-502, and M-437)

Hybrid origin*	Number of crossed spore pairs	Number of zygotes	Number of obtained tetrads	Ascospore viability (%)	Number of the studied tetrads with 2 : 2** segregation
MUCL 30909 × VKM Y-502	32	3	43	38	4
Dji2 × S288C	–	–	15	88	11
YO 495 × X2180-1A	37	8	25	87	14
YO 504 × X2180-1A	37	2	24	98	21
YO 614 × X2180-1A	38	13	48	83	20
CBS 403 × M-437	30	3	21	90	15
CBS 405 ×M-437	35	5	22	83	13
No. 26-1-10 × S288C	–	–	34	82	11
No. 34 × S288C	–	–	34	87	21
No. 127 × X2180-1A	–	–	5	65	3
No. 134 × S288C	–	–	35	92	25

Notes: * The hybrids of strains Dji2, nos. 26-1-10, 34, 127, and 134 were obtained by mass crossing of haploid cells; other hybrids were obtained using the spore-to-spore or spore-to-haploid cell methods.

** For the hybrids of strains MUCL 30909, segregation for the *ADE* gene is shown; for strain Dji2 segregation for the *GAL2* gene is demonstrated; for strains YO 495, YO 504, and YO 614 segregation for the *GAL2* and *MEL* genes is demonstrated; for strains CBS 403 and CBS 405 segregation for the *MAL* gene is shown

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