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EXPERIMENTAL ARTICLES

# Action Spectrum of *Kluyveromyces lactis* Mycocins

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**Abstract**—New mycocinogenic strains of the yeast *Kluyveromyces lactis* were found. They have fungicidic activity at pH from 5 to 7. This activity was eliminated by UV irradiation. Among over 260 species tested, ones sensitive to these mycocins were revealed mainly in the families Saccharomycetaceae and Wickerhamomycetaceae of the order *Saccharomycetales*.

*Keywords*: mycocin (killer toxin), sensitivity, Saccharomycetaceae, Wickerhamomycetaceae

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Secretion of antifungal proteins (mycocinogeny) is a phenomenon widespread among the members of most yeast genera [1]. Mycocinogeny has only been deeply studied in the case of rather few species, primarily *Saccharomyces cerevisiae* Meyen ex Hansen [2]. In other species, only certain aspects of mycocinogeny—genetic determinants of mycocin (killer toxin) synthesis, its structure, or its mechanism of action—were investigated thoroughly. The information concerning one of the principle characteristics of mycocins, their action spectra, is usually poor. A limited selection of cultures is often used, mainly of the species important for medicine or food industry.

The above applies to *Kluyveromyces* species, many of which are known to produce mycocins [3, 4]. Most of the works are concerned with *K. lactis* (Dombrowski) van der Walt, specifically with investigation of the linear DNA of the plasmids responsible for the mycocin (zymocin) synthesis in this species [5].

Our earlier investigation of the yeasts which vigorously ferment lactose revealed the cultures of these species exhibiting antifungal activity [6]. In the present work, the results of investigation of this activity are presented, with special emphasis for revealing of the yeast taxa sensitive to the agents secreted by *K. lactis*.

## MATERIALS AND METHODS

**Strains.** The cultures from the Russian Collection of Microorganisms (VKM) were used. Most of the yeast species were represented by their type strains, while for most genera their type species were used. The active strains *K. lactis* VKM Y-869 and Y-870 were isolated, respectively, from sour cow's milk (Kola Peninsula, in 1937) and chal (Turkmen SSR, in 1948), while strains VKM Y-1186 and VKM Y-1343 were isolated from cow's milk (Belarussian SSR, in 1947 and 1966) (http://www.vkm.ru).

**Assay for sensitivity to mycocins.** Three-day cultures grown on malt agar (MA) was determined at room temperature (~20°C) by the "culture against culture" method. Aqueous suspensions (0.05 mL, $10^5$ cells/mL) of each strain examined for sensitivity were spread over the surface of the agar medium of specific composition (see below). The four *K. lactis* strains mentioned above were then streak inoculated on the plate. The plates were then incubated until growth of the lawn strains appeared. The strains were registered as resistant, weakly sensitive, or sensitive, respectively in the case of no growth inhibition, growth inhibition zone <1 mm, and growth inhibition zone several mm wide.

**Isolation and characterization of the toxin.** To obtain the toxin, *K. lactis* strains were grown in liquid medium without shaking. The cells were separated by centrifugation (5000 *g*, 10 min) and the supernatant was filtered through GF/A glass fiber filters (Sigma, United States). The toxin-containing culture liquid was used for determination of the fungicidal effect. Cell viability was measured after plating on MA. The culture liquid was also used to determine the effect of high temperature and proteolysis on the toxin by the agar well method. For approximate determination of the molecular mass of the toxin, strain VKM Y-1186 was grown on the medium for sensitivity tests covered with a dialysis membrane (Spectrum, United States). After seven days of incubation, the membrane with the culture was removed and the plate was lawn-inoculated with toxin-sensitive strain of *S. bayanus* Saccardo VKM Y-443.

**Elimination of the antifungal activity.** Aqueous suspensions of *K. lactis* strains VKM Y-780, Y-1186, and Y-1343 (0.1 mL) were plated on MA and incubated at the maximal growth temperatures (36, 37, and 41°C,

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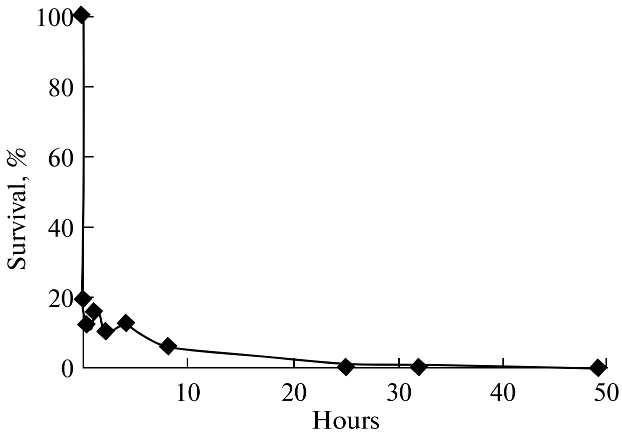


Killing of *Saccharomyces bayanus* VKM Y-443 cells (% of the original concentration, $2.2 \times 10^3$ cells/mL) incubated in toxin-containing culture liquid of *Kluyveromyces lactis* VKM Y-1186.

respectively). Other plates were UV-irradiated for 3 min.

## RESULTS

Antifungal activity of *K. lactis* strains VKM Y-869, Y-870, Y-1186, and Y-1343 was observed at pH 5.0–7.0 (citrate–phosphate buffer). The broadest zones of inhibition of the sensitive cultures were found at pH 5.5 and 6.0; they increased on the medium supplemented with glycerol (150 mL/L) or NaCl (2%). The sensitivity of most of the cultures to the toxins secreted by these strains was therefore tested on the medium containing the following (g/L): glucose, 5.0; peptone, 2.5; yeast extract, 2.0; NaCl, 20.0; agar, 20.0; pH 5.5 and 6.0. According to the size of the growth inhibition zones, strain VKM Y-1186 was the most active one, with VKM Y-1343 being close to it in activity. Two other strains, especially VKM Y-869, exhibited low activity. In the latter case, activity against the cultures weakly sensitive to the toxins of all four *K. lactis* strains, including VKM Y-1186, was sometimes not revealed.

To obtain the toxin, strain VKM Y-1186 was grown for three days in the medium containing the following: glucose, 40.0 g; peptone, 20.0 g; yeast extract, 10.0 g; glycerol, 150 mL; water, to 1 L. To obtain the active toxin-containing culture liquid of strain VKM Y-869, the incubation was as long as 20 days.

The agent secreted by strain VKM Y-1186 was completely inactivated after 5 min incubation at 100°C, as well as by treatment with pepsin, pronases E and P (Serva, Germany), or proteases XIII and XIV from *Streptomyces griseus* and *Aspergillus saitoi* (Sigma, United States). It did not pass through the dialysis membrane impermeable to compounds with molecular masses of 6–8 kDa or more.

Incubation of the sensitive yeasts in toxin-containing culture liquid resulted in a 80–90% drop in the number of viable cells after 30 min, with a subsequent less steep decrease, so that no viable cells were detected after 48 h (figure).

Antifungal activity was examined for 223 randomly chosen colonies of *K. lactis* strains grown at the maximal growth temperatures. Only in the case of strain VKM Y-1186 activity was lost in 14% of the colonies, with 8% becoming sensitive to the toxin of the parent strain. All 128 examined colonies of strains VKM Y-870 and VKM Y-1343 retained activity after cultivation at maximal temperatures.

Among 166 colonies of strains VKM Y-870, VKM Y-1186, and VKM Y-1343 examined after UV irradiation, 83 to 91% showed no antifungal activity. In the case of VKM Y-870 and VKM Y-1343, all colonies were of the neutral phenotype, while among the VKM Y-1186 colonies 32% of the clones which lost activity became sensitive to the toxin of the parent strain.

The sensitivity of yeasts (584 strains of 268 species and 105 genera) to the agents secreted by *K. lactis* was determined. Apart from type strains of one species of *Rhodotorula* Harrison and two species of *Sporobolomyces* Kluyver et van Niel, which exhibited weak sensitivity to the toxins, 95 species (127 strains) of 42 basidiomycetous yeast genera were insensitive (Table 1).

Among the 173 species (457 strains) of 63 ascomycetous yeast genera, *Kluyveromyces* van der Walt species were characterized by sensitivity (Table 2). Certain sensitive species were found within the genera *Barnettozyma* Kurtzman et al., *Kuraishia* Yamada et al., *Metschnikowia* Kamienski, *Nakazawaea* Yamada et al., *Saccharomyces* Meyen ex Reess, *Sporopachydermia* Rodrigues de Miranda, *Trichomonoascus* Jackson emend. Kurtzman et Robnett, *Wickrhamomyces* Kurtzman et al., and *Zygosaccharomyces* Barker (Table 3, includes the type strains of the presently accepted species), as well as in the genera *Candida* Berkhout, *Lindnera* Kurtzman et al., *Ogataea* Yamada et al., and *Schwanniomyces* Klöcker emend. Suzuki et Kurtzman (Table 1).

Almost all these genera were heterogeneous, with both sensitive and resistant strains (Tables 2 and 3). Moreover, heterogeneity in respect to *K. lactis* toxins was found within species as well (Tables 2, 4, and 5).

## DISCUSSION

Thermolability, sensitivity to proteolysis, and significant molecular mass of the fungicidal toxin secreted by *K. lactis* indicate its proteinaceous nature, so this agent may be identified as a mycocin [1]. Since the antifungal activity was efficiently eliminated and sensitivity to the toxin of the parent strain emerged in some cases, mycocin synthesis was likely determined by extrachromosomal genetic elements. It was previously shown that a similar mycocinogenic strain *K. lactis* IFO 1267 (= ATCC 8585, CBS 2359) carries

**Table 1.** Yeast insensitive to *Kluyveromyces lactis* mycocins (number of investigated species, strains)

| | | |
|---|---|---|
| *Agaricostilbum* (1, 1) | *Komagataella* (1, 1) | *Schizoblastosporion* (1, 15) |
| *Ambrosiozyma* (1, 1) | *Kondoa* (1, 1) | *Schizosaccharomyces* (1, 1) |
| *Bensingtonia* (1, 1) | *Kregervanrija* (1, 1) | *Schwanniomyces* (5, 6)* |
| *Blastobotrys* (2, 3) | *Kurtzmanomyces* (1, 1) | *Sebacina* (1, 1) |
| *Brettanomyces* (4, 17) | *Kwoniella* (1, 1) | *Sirobasidium* (1, 1) |
| *Bullera* (1, 1) | *Leucosporidiella* (1, 1) | *Sporidiobolus* (4, 4) |
| *Bulleromyces* (1, 1) | *Leucosporidium* (1, 1) | *Sporobolomyces* (13, 19)* |
| *Candida* (4, 5)* | *Lindnera* (10, 35)* | *Starmera* (4, 7) |
| *Citeromyces* (1, 1) | *Lipomyces* (3, 7) | *Sterigmatomyces* (1, 1) |
| *Clavispora* (1, 2) | *Magnusiomyces* (1, 1) | *Sugiyamaella* (1, 1) |
| *Cryptococcus* (1, 1) | *Mastigobasidium* (1, 1) | *Sympodiomycopsis* (1, 1) |
| *Cuniculitrema* (1, 1) | *Millerozyma* (1, 1) | *Tausonia* (2, 2) |
| *Curvibasidium* (1, 1) | *Mrakia* (1, 1) | *Tetrapisispora* (2, 2) |
| *Cystofilobasidium* (1, 1) | *Mrakiella* (1, 1) | *Tilletiopsis* (1, 1) |
| *Debaryomyces* (1, 1) | *Myxozyma* (1, 1) | *Torulaspora* (4, 4) |
| *Dioszegia* (1, 1) | *Nadsonia* (2, 10) | *Tremella* (1, 1) |
| *Dipodasopsis* (1, 1) | *Naumovozyma* (1, 1) | *Trichosporon* (1, 1) |
| *Eremothecium* (1, 1) | *Ogataea* (10, 13)* | *Trigonopsis* (1, 1) |
| *Erythrobasidium* (1, 1) | *Pachysolen* (1, 1) | *Trimorphomyces* (1, 1) |
| *Fellomyces* (1, 1) | *Peterozyma* (1, 1) | *Udeniomyces* (1, 1) |
| *Fibulobasidium* (1, 1) | *Pichia* (1, 1) | *Vanderwaltozyma* (1, 1) |
| *Filobasidiella* (1, 1) | *Pseudozyma* (1, 1) | *Wickerhamia* (1, 1) |
| *Filobasidium* (1, 1) | *Rhodosporidium* (8, 24) | *Wickerhamiella* (1, 1) |
| *Geotrichum* (1, 1) | *Rhodotorula* (28, 36)* | *Xanthophyllomyces* (1, 1) |
| *Hanseniaspora* (1, 1) | *Saccharomycodes* (1, 5) | *Yamadazyma* (1, 1) |
| *Holtermannia* (1, 1) | *Saccharomycopsis* (1, 1) | *Yarrowia* (1, 1) |
| *Itersonilia* (1, 1) | *Sakaguchia* (1, 1) | *Zygoascus* (1, 1) |
| *Kockovaella* (1, 1) | *Saturnispora* (1, 1) | *Zygotorulaspora* (2, 2) |
| *Kodamaea* (1, 1) | *Scheffersomyces* (1, 1) | |

Note: * Some strains, including the type ones, of the species *C. glabrata, C. holmii, C. intermedia*, and *C. milleri* (see also Table 2), *L. fabianii, L. jadinii, L. petersonii, O. nonfermentans, O. pini, O. wickerhamii, Rh. hordea, Schw. occidentalis, Schw. etchelsii, Sp. phyllomatis*, and *Sp. salmoneus* exhibited weak sensitivity. They are not listed among the numbers of strains and species given in the table.

two double-stranded DNA plasmids, one of which encodes the synthesis of a three-subunit glycoprotein with molecular mass ~150 kDa [5].

The mechanism of action of this mycocin with endoribonuclease and chitinase activity [7] is presently under investigation [8]. The spectrum of sensitive species remained poorly characterized, so that only 13 ascomycetous species (considering synonymy) were investigated [9]. Low representativity, as well as strain differences in the sensitivity within species, make it difficult to compare these results with our data.

According to our results and considering the weak activity of strain VKM Y-869, the action spectra of the extracellular agent of four investigated *K. lactis* strains, while qualitatively identical, differed significantly in level of activity.

Unlike most *Kluyveromyces* strains, some strains of this species were resistant to the mycocins (Table 2), while some *S. cerevisiae* and *W. anomalus* strains were sensitive (Tables 3 and 4). These *Saccharomyces* strains exhibited high DNA–DNA homology to the neotype strain of *S. cerevisiae* [10], so this heterogeneity is probably an intraspecific feature. The sensitivity of *S. cerevisiae* strains to *K. lactis* mycocin was found to depend on the mating type and ploidy of the cultures [5, 11]. In diploid and polyploid strains it was significantly lower than in haploid ones.

Insensitivity of *K. lactis* strains, including its anamorph *C. sphaerica*, results probably from their immunity to its own mycocin, which is responsible for the neutral phenotype. The lack of sensitivity in some strains of a related species *K. marxianus* (anamorph

**Table 2.** Intrageneric spectra of *Kluyveromyces lactis* mycocins

| Species and strains | Mycocinogenic strains | | |
|---|---|---|---|
| | 869 | 870 | 1186<br>1343 |
| *Kluyveromyces aestuarii* (Fell) van der Walt BKM Y-1528T | w | w | w |
| *K. dobzhanskii* (Shehata et al.) van der Walt | | | |
| BKM Y-1293T (*Saccharomyces dobzhanskii*, T) | w | + | + |
| BKM Y-2744 (*Kluyveromyces marxianus* var. *dobzhanskii*) | – | w | w |
| BKM Y-2745 (*K. marxianus* var. *dobzhanskii*) | w | + | + |
| *K. lactis* (Dombrowski) van der Walt var. *lactis* | | | |
| BKM Y-854 (*Zygosaccharomyces casei*, T) | w | w | w |
| BKM Y-868NT (*Zygosaccharomyces lactis*) | – | w | w |
| BKM Y-896 (*Z. versicolor*, T) | w | w | w |
| BKM Y-1527 (*Saccharomyces sociasi*, T) | w | w | w |
| *Candida sphaerica* (Hammer et Cordes) Meyer et Yarrow | | | |
| BKM Y-762T (*Torulopsis sphaerica*) | – | – | – |
| *Kluyveromyces lactis* (Dombrowski) van der Walt var. *drosophilarum* | | | |
| (Shehata et al.) Sidenberg et Lachance | | | |
| BKM Y-831 (*Zygofabospora krassilnikovii,* T) | w | w | w |
| BKM Y-1296 (*Saccharomyces phaseolosporus*, T) | – | – | – |
| BKM Y-1302T (*S. drosophilarum*) | w | + | + |
| BKM Y-1535 (*Kluyveromyces vanudenii*, T) | – | w | w |
| *K. marxianus* (Hansen) van der Walt | | | |
| BKM Y- 486 (*Saccharomyces muciparus*, T) | – | – | – |
| BKM Y-848 (*Zygosaccharomyces ashbyi*, T) | w | w | w |
| BKM Y-876NT (*Z. marxianus*) | – | – | – |
| BKM Y-1187 (*Saccharomyces macedoniensis,* A) | – | – | – |
| BKM Y-1242 (*S. fragilis* var. *bulgaricus*, T) | w | w | w |
| BKM Y-1517 (*Kluyveromyces cicerisporus*, T) | w | + | + |
| BKM Y-1545 (*K. wikenii*, T) | – | – | – |
| *Candida kefyr* (Beijerinck) van Uden et Buckley | | | |
| BKM Y-48 (*C. macedoniensis*, T) | – | – | – |
| BKM Y-257T (*Mycotorula kefyr*) | – | – | – |
| BKM Y-258 (*M. lactis*) | – | + | + |
| BKM Y-434 (*Saccharomyces fragrans*, T) | – | + | + |
| BKM Y-922 (*Mycotorula lactosa*, T) | w | w | + |
| *Kluyveromyces wickerhamii* (Phaff et al.) van der Walt | | | |
| BKM Y-1297T (*Saccharomyces wickerhamii*) | – | w | w |

Note: "+", "w", and "–" indicate sensitivity, weak sensitivity, and insensitivity, respectively. T, NT, and A stand for type, neotype, and authentic strains. The original names of the strains are given in parentheses.

**Table 3.** Action spectra of *Kluyveromyces lactis* mycocins against ascomycetous yeasts

| Species (number of strains investigated) | Mycocinogenic strains, VKM Y- | | | |
|---|---|---|---|---|
| | 869 | 870 | 1186 | 1343 |
| *Aciculoconidium aculeatum* (1) | + | w | w | w |
| *Babjeviella inositovora* (1) | – | w | w | w |
| *Barnettozyma californica* (7) | – | w | w | w |
| *B. pratensis* (4) | w | + | + | + |
| *B. salicaria* (1) | – | – | – | – |
| *Hyphopichia burtonii* (1) | – | w | w | w |
| *H. heimii* (1) | – | w | w | w |
| *Kazachstania africana* (1) | – | – | – | – |
| *K. barnettii* (1) | – | – | – | – |
| *K. bovina* (1) | w | w | + | + |
| *K. exigua* (2) | – | – | – | – |
| *K. kunashiriensis* (1) | – | w | w | w |
| *K. lodderae* (1) | – | w | w | w |
| *K. rosinii* (1) | – | – | – | – |
| *K. sloofiae* (1) | – | – | – | – |
| *K. spencerorum* (2) | – | – | – | – |
| *K. transvaalensis* (1) | – | – | – | – |
| *K. unispora* (2) | – | w | w | w |
| *K. viticola* (1) | – | w | w | w |
| *K. yakushimaensis* (1) | – | – | – | – |
| *Kuraishia capsulata* (2) | w | + | w | + |
| *Lachancea thermotolerans* (1) | – | w | + | w |
| *L. waltii* (1) | – | – | – | – |
| *Lodderomyces elongisporus* (1) | w | w | + | w |
| *Metschnikowia agaweae* (1) | – | – | – | – |
| *M. australis* (1) | w | w | + | + |
| *M. bicuspidata* (3) | w | w | + | w |
| *M. bicuspidata* var. *kamienskii* (1) | – | – | – | – |
| *M. gruessii* (1) | – | – | – | – |
| *M. krissii* (1) | w | w | + | w |
| *M. lunata* (1) | w | w | w | w |
| *M. pulcherrima* (1) | – | – | – | – |
| *M. reukafii* (1) | – | – | – | – |
| *M. zobellii* (1) | w | + | + | + |
| *Meyerozyma guilliermondii* (4) | – | w | w | w |
| *Nakazawaea holstii* (5) | w | w | + | w |
| *Nakaseomyces delphensis* (1) | + | + | + | + |
| *Priceomyces carsonii* (6) | – | w | + | + |
| *P. castillae* (1) | – | w | w | w |
| *P. haplophilus* (1) | – | w | + | + |
| *P. medius* (2) | – | w | w | w |
| *P. melissophilus* (1) | – | – | – | – |
| *Saccharomyces bayanus* (7) | w | + | + | + |
| *S. cariocanus* (2) | – | – | – | – |
| *S. cerevisiae* (40) | – | – | – | – |

**Table 3.** (Contd.)

| Species (number of strains investigated) | Mycocinogenic strains, VKM Y- | | | |
|---|---|---|---|---|
| | 869 | 870 | 1186 | 1343 |
| *S. kudriavzevii* (2) | – | – | – | – |
| *S. mikatae* (2) | – | – | – | – |
| *S. paradoxus* (8) | – | – | – | – |
| *S. pastorianus* (2) | – | – | – | – |
| *Sporopachydermia lactativora* (1) | – | w | w | w |
| *Trichomonoascus ciferrii* (1) | w | + | + | + |
| *Wickerhamomyces alni* (2) | w | w | + | + |
| *W. anomalus* (29)* | – | – | – | – |
| *W. bisporus* (1) | w | w | w | w |
| *W. bovis* (1) | w | w | w | w |
| *W. canadensis* (1) | w | + | + | + |
| *W. chambardii* (1) | w | w | + | w |
| *W. ciferrii* (1) | w | w | + | w |
| *W. lynferdii* (1) | – | w | + | + |
| *W. mucosus* (1) | – | w | w | w |
| *W. pijperi* (1) | – | – | – | – |
| *W. rabaulensis* (1) | w | w | + | w |
| *W. silvicola* (1) | – | – | – | – |
| *W. strasburgensis* (2) | w | w | + | w |
| *W. subpelliculosa* (6) | w | w | w | w |
| *W. sydowiorum* (1) | + | w | w | w |
| *Zygosaccharomyce bailii* (2) | w | + | + | + |
| *Z. bisporus* (1) | – | – | – | – |
| *Z. kombuchaensis* (1) | – | – | – | – |
| *Z. mellis* (2) | w | w | w | w |
| *Z. rouxii* (12) | w | w | + | + |

Note: * 26 strains were weakly sensitive.

*C. kefyr*), which some authors consider a variety of *K. lactis* [10], may be due to the same reason.

Among the reasons of variability in the sensitivity of *Zygosaccharomyces* species to *K. lactis* mycocins (Tables 3 and 5), taxonomic heterogeneity should not be ruled out. In these cases, synonymy is presently based on the similarity of their phenotypic characteristics alone [10]. Molecular biological data suggest that the presently accepted taxa may be complexes of closely related species [12, 13].

Interestingly, aquatic *Metschnikowia* species, which unlike terrestrial ones were sensitive to the mycocins of *Pichia membranifaciens* (Hansen) Hansen [14], differed also in their sensitivity to *K. lactis* mycocins (Table 3).

In general, our results make it possible to conclude that all ascomycete yeasts sensitive to *K. lactis* mycocins belong to the order Saccharomycetales, most of them being members of the families Saccharomycetaceae and Wickerhamomycetaceae (Tables 2 and 3).

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**Table 4.** *Saccharomyces cerevisiae* strains sensitive to *Kluyveromyces lactis* mycocins

| Species, strains (original names) | Mycocinogenic strains | | |
|---|---|---|---|
| | 869 | 870<br>1343 | 1186 |
| BKM Y-388 (*S. anamensis* Will et Heinrich, T) | – | w | w |
| BKM Y-389 (*S. batatae* Saito, A) | – | w | w |
| BKM Y-390 (*S. ellipsoideus* Reess var. *fulliensis* Steiner, T) | w | w | w |
| BKM Y-391 (*S. cerevisiae* Hansen subsp. *orsati* Steiner, T) | – | w | w |
| BKM Y-393 (*S. cerevisiae* Hansen subsp. *vetrozensis* Steiner, T) | w | w | w |
| BKM Y-402 (*S. festinans* Ward et Baker, T) | w | + | + |
| BKM Y-403 (*S. marshalianus* Kufferath, T) | – | w | w |
| BKM Y-404 (*S. cerevisiae* Hansen var. *onychophilius* Zach, T) | w | w | w |
| BKM Y-406 (*S. chevalieri* Guillermond, T) | – | w | w |
| BKM Y-407 (*S. chevalieri* Guillermond var. lindneri (Guillermond) Dekker, T) | w | w | w |
| BKM Y-424 (*S. ellipsoideus* Reess var. *umbra* Castelli, T) | w | w | w |
| BKM Y-441 (*S. hutensis* Kufferath *ex* Stelling-Dekker, T) | w | + | + |
| BKM Y-1144 (*S. pulmonalis* Redaelli, T) | w | + | + |
| BKM Y-1234 (*S. oleaginosus* Santa Maria, T) | w | w | + |
| BKM Y-2119 (*S. gaditensis* Santa Maria, T) | w | + | + |

**Table 5.** *Zygosaccharomyces* strains insensitive to *Kluyveromyces lactis* mycocins

| Species, strains (original names) |
|---|
| *Zygosaccharomyces bailii* (Lindner) Guilliermond |
| BKM Y-419 (*Saccharomyces elegans* Lodder et Kreger-van Rij, T) |
| BKM Y- 644 (*Saccharomycodes mestris* Marcilla et Feduchy, T) |
| BKM Y- 874 (*Zygosaccharomyces mandchuricus* Saito, T) |
| *Z. mellis* Fabian et Quinet |
| BKM Y-878 (*Zygosaccharomyces mellis-acidi* von Richter, T) |
| BKM Y-883 (*Z. nussbaumeri* Lochhead et Heron, T) |
| BKM Y-885 (*Z. perpicillatus* Sacchetti, T) |
| *Z. rouxii* (Boutroux) Yarrow |
| BKM Y-861 (*Zygosaccharomyces felsineus* Sacchetti, T) |
| BKM Y-872 (*Z. salsus* Takahashi et Yukawa, T) |
| BKM Y-873 (*Z. major* Takahashi et Yukava var. *threntensis* Lodder, T) |
| BKM Y-882 (*Z. nectarophilus* Lochhead et Farrell, T) |
| BKM Y-891 (*Z. richteri* Lochhead et Heron, T) |

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