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MYCOCINOGENY IN FISSION YEAST

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A total of 39 *Schizosacchromyces* strains have been examined for antagonistic activity, including the nomenclature types of the species with the names considered presently synonymous. Two strains of *S. pombe* exhibited fungistatic activity. They are not active against any budding yeasts (*Saccharomycotina*) but act against some species of the genera *Protomyces* and *Taphrina* (*Taphrinomycotina*).

Key words: killer toxin, mycocin, phylogeny, *Schizosaccharomyces*

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Some strains of many ascomycetous and basidiomycetous yeast species secrete (glyco)proteins (mycocins, killer toxins) having fungicidal or fungistatic action. A molecular mass of mycocins may range from 10 to more 100 kDa. The mechanisms of their actions are different: they cause membrane damage, an arrest in the G1 phase of the cell cycle, an inhibition of DNA or glucan synthesis. The genetic determinants of mycocins can be either chromosomal or cytoplasmical. In the second case, dsRNA viruses or linear dsDNA plasmids are responsible for mycocinogeny. In spite of these varieties, one characteristic remains the same, namely, sensitivity to mycocins is restricted to organisms phylogenetically related to the mycocinogenic strains (Golubev, 2006). The degree of relatedness may be different owing to the diversity of cell-wall receptors involved in binding of mycocins that can be both unique and common for certain taxa.

From this viewpoint the communications about activity against organisms distantly related to mycocinogenic strains cast doubt. As an example, killer activity of *Schizosaccharomyces pombe* Lindner against *Candida glabrata* (Anderson) Meyer et Yarrow and *Saccharomyces cerevisiae* Meyen ex Hansen has been reported (Bonillia-Salinas et al., 1995), though budding yeasts belong to Saccharomycotina subphylum whereas fission yeasts do to "*Archiascomycotina*" (Kuramae et al., 2006). This inconsistency initiated a search of mycocinogenic strains of *Schizosaccharomyces.*

Strains from All-Russian Collection of Microorganisms (VKM) (http://www.vkm.ru) and Raiffeisen-Bio-Forschung (RBF, Austria) were used in the present work. Sensitivity of three day cultures grown on malt agar was determined at room temperature using the "culture against culture" method. Water suspensions (0.05 mL, 10^5 cells/mL) were spread over the surface of a buffered (with citrate-phosphate buffer) medium containing the following (g/L): glucose, 5.0; peptone, 2.5; yeast extract, 5.0; agar, 20.0. The cultures of *Schizosaccharomyces pombe* were then streak inoculated. The plates were incubated until cell lawns developed. The strains forming growth inhibition zones several mm wide, zones ~1 mm wide, and no zones were recorded as sensitive, weakly sensitive, and insensitive, respectively.

When screening 39 strains of the genus *Schizosaccharomyces* (three species) for mycocin secretion, two strains of *S. pombe*, VKM Y-1876 and VKM Y-1912, received as *S. acidodevoratus*, were found that they show the activity (Table 1). They exhibited antagonistic activity at pH from 3.5 to 5.5 (citrate-phosphate buffer). At pH 6.0 the activity did not revealed. Growth inhibition zones were the most pronounced at pH 4.0. Zones became broader on the media supplemented with glycerol (100 ml/l). Five strains of *S. pombe* were sensitive, the rest strains of this species as well as *S. japonicus* and *S. octosporus* have neutral phenotype. Blue edging has not developed on the lawns of sensitive strains along the streaks of mycocinogenic *S. pombe* strains on the medium with methylene blue (0.03 g/l) that indicated fungistatic action of secreted agents.

The two mycocinogenic strains are not active against any representatives of Saccharomycotina belonging to 13 species of 12 genera but they act against *Protomyces macrosporus*, *Taphrina bergeniae*, *T. carnea* and *T. tosquinetii* (Table 2). All strains of *Schizosaccha-*

Table 1. Intrageneric activity of *Schizosaccharomyces pombe* mycocinogenic strains

Species, strains (original names)	1	2
Schizosaccharomyces japonicus Yukawa et Maki		
VKM Y-651^{T}, 655, 656, 667	–	–
VKM Y-668 (*S. versatilis* Wickerham et Duprat)	–	–
S. octosporus Beijerinck		
VKM Y-654^{NT}	–	–
VKM Y-2195 (*S. sloofiae* Kumbhojkar, T)	–	–
S. pombe Lindner		
VKM Y-646 (*S. acidodevoratus* Chalenko, A)	–	–
VKM Y-647, 648, 1895 (*S. acidodevoratus* Chalenko)	w	w
VKM Y-649 (*S. formosensis* Nakazawa var. *tapaniensis* Nakazawa, T)	–	–
VKM Y-650 (*S. hominis* Benedek)	+	+
VKM Y-652 (*S. mellacei* Jörgensen)	–	–
VKM Y-653 (*S. mosquensis* Shcherbakov et Popova)	–	–
VKM Y-657 (*S. pinan* Nakazawa)	–	–
VKM Y-658^{NT} 662	–	–
VKM Y-663 (*S. liquefaciens* Osterwalder, T)	–	–
VKM Y-664 (*S. santawensis* Nakazawa, T)	–	–
VKM Y-665 (*S. taito* Nakazawa, T)	–	–
VKM Y-669 (*S. vordermani* Wehmer)	–	–
VKM Y-1349-1 – 1349-3 (*Quadrisporomyces adherents* Sekunova, A)	–	–
VKM Y-1580 (*S. mosquensis* Shcherbakov et Popova, A)	–	–
VKM Y-1874, 1875, 1913, 1914, 1916, 2543	–	–
VKM Y-1921 (*S. acidodevoratus* Chalenko)	+	+
VKM Y-2051 (*S. malidevorans* Rankine et Fornachon, T)	–	–

Note: 1 – VKM Y-1876; 2 – VKM Y-1912; "+" – sensitive; w – weakly sensitive; "–" – non-sensitive; T – type strain; NT – neotype strain; A – author's strain. Original names were presented in the parentheses.

romyces spp. examined are insensitive to mycocin produced by type strain of *Lachancea* (= *Kluyveromyces*) *waltii* (Kono, Himeno, 1997).

The differences between yeast fungi in mycocin sensitivity patterns are correlated with whole range of taxonomic and phylogenetic markers, such as cell ultrastructure, composition of polysacchardes, sequence

Table 2. Action spectrum of *Schizosaccharomyces pombe* mycocins

Species, strains	VKM Y-1876	VKM Y-1912
Candida glabrata (Andersen) Meyer et Yarrow VKM Y-732, 1481^{T}	–	–
C. stellata (Kroemer et Krumb.) Meyer et Yarrow VKM Y-763^{T} 2575	–	–
Citeromyces matritensis (Santa Maria) Santa Maria VKM Y-1247	–	–
Dekkera bruxelensis van der Walt VKM Y-20	–	–
Geotrichum fermentans (Diddens et Lodder) von Arx VKM Y-813	–	–
Hanseniaspora valbyensis Klöcker VKM Y-138T	–	–
Kazachstania viticola Zubkova VKM Y-1659T	–	–
Kluyveromyces marxianus (Hansen) van der Walt VKM Y-876^{NT}	–	–
Pichia membranifaciens (Hansen) Hansen VKM Y-299^{T}	–	–
Protomyces macrosporus Unger VKM F-2977	+	+
Saccharomyces cerevisiae Meyen ex Hansen VKM Y-388, 390, 391, 402, 403, 406, 407, 424, 1144	–	–
Saccharomycodes ludwigii (Hansen) Hansen VKM Y-626	–	–
Taphrina bergeniae Döbbeler VKM F-2965	+	+
T. betulina Rostrup RBF 659	–	–
T. carnea Johanson RBF 662	+	w
T. deformans (Berkeley) Tulasne RBF 672	–	–
T. pruni Tulasne RBF 688	–	–
T. purpurescens Robinson RBF 690	–	–
T. tosquinette (Westend.) Tulasne RBF 699	+	+
Totulaspora delbrueckii (Lindner) Lindner VKM Y-708	–	–
Zygosaccharomyces bailii (Lindner) Guilliermond VKM Y-419, 850	–	–

similarity of small and large subunit ribosomal RNAs (Golubev, 2012). In most cases, the strains of the same species have identical responses to specific mycocins. As a rule, teleomorphic taxa are homogenous, unlike the anamorphic taxa in which heterogeneity in mycocin sensitivity patterns is much more widespread.

The data presented further support placement of *Protomyces*, *Schizosaccharomyces* and *Taphrina* at the base of *Ascomycota* as the "*Archiascomycetes*" (the subdivision *Taphrinomycotina*).

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Микоциногения у делящихся дрожжей

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На наличие антагонистической активности обследовано 39 штаммов *Schizosaccharomyces*, включая типовые штаммы видов, наименования которых в настоящее время рассматриваются как синонимы. Фунгистатическую активность проявляли два штамма *Schizosaccharomyces pombe.* Они неактивны против почкующихся дрожжей (*Saccharomycotina*), но действуют против некоторых видов *Protomyces* и *Taphrina* (*Taphrinomycotin*a).

Ключевые слова: киллер-токсины, микоцины, филогения, *Schizosaccharomyces*