ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 2, pp. 154–157. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © W.I. Golubev, 2011, published in Mikrobiologiya, 2011, Vol. 80, No. 2, pp. 166–168.

EXPERIMENTAL ARTICLES

Differentiation between Aquatic and Terrestrial *Metschnikowia* Species of Based on Their Sensitivity to *Pichia membranifaciens* Mycocins

W. I. Golubev[1]

Russian Collection of Microorganisms, Skryabin Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia

Received May 13, 2010

Abstract—Aquatic *Metschnikowia* species (*M. australis, M. bicuspidata, M. krissii*, and *M. zobellii*) are sensitive to 10 out of 12 mycocins secreted by *Pichia membranifaciens* strains. Terrestrial species *M. pulcherrima* and *M. reukaufii* are resistant to all these mycocins, while *M. gruessii* and *M. lunata* are sensitive to one of them. The yeast described as *Torula rubifaciens* is also sensitive to this mycocin.

Keywords: mycocin, killer toxin, taxonomy, ecology, *Pichia, Metschnikowia*.

DOI: 10.1134/S0026261711010085

Yeasts of the genus *Metschnikowia* Kamienski producing needle-shaped ascospores are divided into two ecological groups. Some of them are found in aquatic habitats and can be isolated from marine and fresh waters, algae, fish, small animals (water fleas, shrimps, and trematodes), to which they are pathogenic. Other species can be revealed in the phyllosphere (flowers and fruits); they are probably facultative pathogens of pollinating insects [1]. These ecological groups differ from each other in their sensitivity to mycocins (killer toxins) produced by group II strains of *Pichia punctispora* (Mélard) Dekker. Unlike aquatic species, terrestrial species are resistant to mycocins [2]. The differences in sensitivity to mycocins, which are taxon-specific [1], are probably not restricted to the mycocins produced by *P. punctispora*.

In this work, aquatic and terrestrial members of the genus *Metschnikowia* were studied with regard to their sensitivity to the fungicidal mycocins produced by *P. membranifaciens* Hansen [3], a close relative of *P.* punctispora.

MATERIALS AND METHODS

In our study, we used strains obtained from the Russian Collection of Microorganisms (VKM, http://www.vkm.ru). Mycocin-producing strains are listed in Table 1. They are grouped according to the action spectra of their mycocins within *P. membranifaciens*, including its anamorph, *Candida valida* (Leberle) van Uden et Buckley. The tests were performed on glucose–peptone medium with glycerol and succinate–sodium or citrate–phosphate buffers (pH 4.5 and 5.0) using the "culture versus culture" method [3]. The sensitivity of the studied cultures to mycocin was determined by the presence of growth inhibition zones surrounding the colonies of mycocin-producing strains on the agar surface. The diagnostic properties of the studied yeast strains were determined on recommended media using standard methods [1].

Table 1. Mycocin-producing strains of *Pichia membranifaciens* grouped on the basis of their intraspecific action spectra [3]

Strains	Groups
VKM Y-166 (*Willia belgica*, T)	VII
VKM Y-242 (*Mycoderma decolorans*, T)	VI
VKM Y-284 (*Pichia alcoholophila*)	VII
VKM Y-299T (*P. membranifaciens*)	VII
VKM Y-843 (*Zygopichia chevalieri*)	VIII
VKM Y-898 (*Pichia chodati* var. *fermentans*, A)	VI
VKM Y-1158 (*P. membranifaciens*)	IX
VKM Y-1159 (*P. membranifaciens* var. *belgica*)	VI
VKM Y-1160 (*P. membranifaciens* var. *belgica*)	IX
VKM Y-1259 (*P. membranifaciens*)	VII
VKM Y-1390 (*P. saccharophila*)	VII
VKM Y-1493 (*Mycoderma valida*, T)	VI

Note: The names under which the cultures were obtained by the Russian Collection of Microorganisms (VKM) are given in parentheses. T, type strain, NT, neotype strains, and A, authentic strains.

[1] Corresponding author; e-mail: wig@ibpm.pushchino.ru

Table 2. Action spectra of the mycocins produced by *Pichia membranifaciens* within the genus *Metschnikowia*

Species, strains	Groups of mycocin-producing strains, VKM Y-				
	843	284	242 1493	166 299 898 1158 1159 1160 1390	1259
Metschnikowia agaves VKM Y-2840T, 2841	–	–	–	–	–
M. pulcherrima VKM Y-4 (*Asporomyces uvae*)	–	–	–	–	–
VKM Y-64 (*Candida pulcherrima*, NT)	–	–	–	–	–
VKM Y-82 (*Cryptococcus interdigitalis*)	–	–	–	–	–
VKM Y-757 (*Torulopsis pulcherrima*)	–	–	–	–	–
VKM Y-758 (*T. pulcherrima* var. *variabilis*, T)	–	–	–	–	–
VKM Y-921, 1208, 1228	–	–	–	–	–
VKM Y-1467 (*Chlamydozyma reukaufii*)	–	–	–	–	–
VKM Y-1468 (*Ch. pulcherrima*)	–	–	–	–	–
VKM Y-1478 (*Torulopsis burgeffiana*)	–	–	–	–	–
VKM Y-1479, 1490, 1955, 2521–2529	–	–	–	–	–
M. reukaufii VKM Y-1466, 1542 (*Chlamydozyma zygota*)	–	–	–	–	–
Candida pulcherrima VKM Y-703 (*Torula rubefaciens*, T)	–	–	–	–	+
M. gruessii VKM Y-2 (*Nectaromyces reukafii*, T), 1591	–	–	–	–	+
M. lunata VKM Y-1651T	–	–	–	–	+
VKM Y-2520 (*Schizoblastosporion kobayashii*, T)	–	–	–	–	+
M. bicuspidata VKM Y-1284 (*M. kamienskii*, T)	–	–	w	+	+
VKM Y-2628HT	–	–	+	+	+
M. bicuspidata var. *californica* VKM Y-2672T	–	–	+	+	+
M. bicuspidata var. *chatamia* VKM Y-2671T	–	–	+	+	+
M. zobellii VKM Y-221T	–	–	+	+	+
M. krissii VKM Y-220T	–	–	+	+	+
M. australis VKM Y-2670T	–	w	+	+	+

Note: Designations: +, sensitive; w, weak sensitivity; –, in sensitive. Other designations: see Table 1.

RESULTS

The grouping of mycocin-producing strains by their action spectra against *Metschnikowia* species differs from the grouping [3] based on the study of only *P. membranifaciens* strains (Table 1). The groups VI and VII determined during the previously performed intraspecific screening split up, and some strains fell into group IX (Table 2).

The action spectra of specific mycocins produced by *P. membranifaciens* strains are different among *Metschnikowia* species. For instance, the mycocin produced by strain VKM Y-843 had no effect on these species, whereas the mycocin produced by strain VKM Y-284 had a weak effect only on *M. australis* (Fell et Hunter) Mendonca-Hagler et al. Other aquatic species and varieties [*M. bicuspidata* (Metschnikov) Kamienski var. *californica* Pitt et Miller, *M. bicuspidata* (Metschnikov) Kamienski var. *chathamia* Fell et Pitt, *M. krissii* (van Uden et Castelo-Branco) van Uden, and *M. zobelii* (van Uden et Castelo-Branco) van Uden)], including the type spe-

cies of the genus, *M. bicuspidata* (Metschnikov) Kamienski, were resistant to this mycocin. The aquatic *Metschnikowia* species were found to be sensitive to all other mycocins produced by *P. membranifaciens* (Table 2). Among all cultures of *M. bicuspidata*, only the type strain *M. kamienskii* Spencer et al. has a weak sensitivity to the mycocins produced by strains VKM Y-242 and VKM Y-1493.

Contrary to the aquatic species, the terrestrial species *M. pulcherrima* Pitt et Miller and *M. reukaufii* Pitt et Miller, widely occurring in nature, are resistant to all mycocins produced by *P. membranifaciens*. The species *M. gruessii* Giménez-Jurado and *M. lunata* Golubev are also resistant to these mycocins, with the exception of the mycocin produced by strain VKM Y-1259 and having the broadest action spectrum (Table 2). The type strain *Torula rubefaciens* Grosbüsch VKM Y-703 (= CBS 2238) is also sensitive to the mycocin of strain VKM Y-1259. Although this strain is listed under the name *M. pulcherrima* in the VKM and CBS (Centraalbureau voor Schimmelcultures) catalogues, we failed to detect either "pulcherrima" cells or ascospores. This strain produces polymorphic cells and is able to synthesize pulcherrimin.

DISCUSSION

The differentiation of the ecological groups within the genus *Metschnikowia* demonstrated in this work corresponds to that based on the sensitivity to *P. punctispora* mycocins, except for the species *M. lunata*, which was grouped with the aquatic species on the basis of its response to these mycocins [2].

The sensitivity to mycocins is determined, first of all, by the presence of mycocin-binding receptors containing glucans, mannans, or chitin on the surfaces of yeast cells [4]. Various *P. membranifaciens* strains produce mycocin sorbed by glucans [5] or mannans [6]. There is evidence that *Metschnikowia* species differ in their cell wall compositions. According to the results of hydrolysate analyses, glucans prevailed in the cell walls of aquatic species; in the cell walls of terrestrial strains, the content of glucans was considerably lower, while the content of mannans was double that in the aquatic strains [7]. These biochemical differences were possible reasons for different responses of the aquatic and terrestrial *Metschnikowia* species to the mycocins produced by *P. membranifaciens* and *P. punctispora*.

The differences between aquatic and terrestrial *Metschnikowia* species in their sensitivity to mycocins (Table 2) correlate with the results of molecular biological investigations, according to which they form different phylogenetic groups [8–10]. Unlike aquatic species, the terrestrial ones produce chlamydospores, so-called "pulcherrima" and "reukafia" cells, which than are transformed into asci. All these morphological, biochemical, and molecular biological characteristics suggest that the genus *Metschnikowia* Kamienski with the type species *M. bicuspidata* should be restricted to aquatic species.

On the basis of the level of DNA–DNA homology (about 90%) [11], *M. kamienskii* is now regarded as a synonym for *M. bicuspidata* var. *bicuspidata* [1]; however, cell hybridization between these species has not been observed [12]. The differences between these species in their sensitivity to mycocins, as well as in their maximum growth temperatures [1], allow us to regard *M. kamienskii* as a variety, i.e., *M. bicuspidata* var. *kamienskii*.

The taxonomic position of *T. rubifaciens*, which is sensitive to the mycocin produced by strain VKM Y-1259, is yet to be determined (Table 2). Its name was previously listed among the synonyms of *C. pulcherrima* (Lindner) Windisch [13]. For this strain, there is no information on the induction of ascospore formation. It differs from *M. gruessii* and *M. lunata* in its ability to produce pulcherrimin, as well as in some morphological and assimilation characteristics.

ACKNOWLEDGMENTS

This work was supported by the program Molecular and Cell Biology of the Presidium of the Russian Academy of Sciences.

REFERENCES

1. *The Yeasts: A Taxonomic Study*, Kurtzman, C.P. and Fell, J.W., Eds., Amsterdam: Elsevier Sci. Publ. B.V, 1998.
2. Golubev, W.I. and Blagodatskaya, V.M., Intra- and Intrageneric Spectra of Action for the Mycocins of *Pichia punctispora* (Mélard 1910) Dekker 1931, *Mikrobiologiya*, 1994, vol. 63, no. 4, pp. 637–642.
3. Golubev, W.I. and Blagodatskaya, V.M., Taxonomic Heterogeneity of *Pichia membranifaciens* Hansen Revealed by Killer-Sensitive Reactions, *Mikrobiologiya*, 1993, vol. 62, no. 2, pp. 291–299.
4. Golubev, W.I., Antagonistic Interactions among Yeasts, in *Biodiversity and Ecophysiology of Yeasts*, Rosa, C.A. and Peter, G., Eds., Berlin: Springer, 2006, pp. 197–219.
5. Santos, A., Marquina, D., Leal, J.A., and Peinado, J.M., (1,6)-β-D-Glucan as Cell Wall Receptor for *Pichia membranifaciens* Killer Toxin, *Appl. Environm. Microbiol.*, 2000, vol. 66, no. 5, pp. 1809–1813.
6. Santos, A., San, MauroM., Bravo, E., and Marquina, D., PMKT2, a New Killer Toxin from *Pichia membranifaciens*, and Its Promising Biotechnological Properties for Control of the Spoilage Yeast *Brettanomyces bruxellensis, Microbiology (UK)*, 2009, vol. 155, no. 2, pp. 624–634.
7. Lopandic, K., Prillinger, H., Molná, O., and Giménez-Jurado, G., Molecular Characterization and Genotypic Identification of *Metschnikowia* Species, *Syst. Appl. Microbiol.*, 1996, vol. 19, no. 3, pp. 393–4026.
8. Mendonç-Hagrler, L.C., Hagler, A.N., and Kurtzman, C.P., Phylogeny of *Metschnikowia* Species Estimated from

Partial rRNA Sequences, *Int. J. Syst. Bacteriol.*, 1993, vol. 43, no. 2, pp. 368–373.

9. Kurtzman, C.P. and Robnett, C.J., Identification and Phylogeny of Ascomycetous Yeasts from Analysis of Nuclear Large Subunit (26S) Ribosomal DNA Partial Sequences, *Antonie van Leeuwenhoek*, 1998, vol. 73, no. 4, pp. 331–371.
10. Giménez-Jurado, G., Kurtzman, C.P., Starmer, W.T., and Spencer-Martins, I., *Metschnikowia vanudenii* sp. nov. and *Metschnikowia lachancei* sp. nov., from Flowers and Associated Insects in North America, *Int. J. Syst. Evol. Microbiol.*, 2003, vol. 53, no. 5, pp. 1665–1670.
11. Mendonça-Hagler, L.C., Hagler, A.H., Phaff, H.J., and Tredic, J., DNA Relatedness among Aquatic Yeasts of the Genus *Metschnikowia* and Proposal of the Species *Metschnikowia australis* comb. nov., *Can. J. Microbiol.*, 1985, vol. 31, no. 10, pp. 905–910.
12. Pitt, J.I. and Miller, M.W., Speciation in the Genus *Metschnikowia, Antonie van Leeuwenhoek*, 1970, vol. 36, no. 3, pp. 357–381.
13. Lodder, J. and Kreger-van Rij, N.J.W., *The Yeasts, A Taxonomic Study*, Amsterdam: North-Holland Publ. Co, 1952.