Metschnikowiella zobellii* sp.nov. and *M. krissii* sp.nov., two Yeasts from the Pacific Ocean Pathogenic for *Daphnia magna

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SUMMARY

Metschnikowiella zobellii sp.nov. and *M. krissii* sp.nov. are described and Latin diagnoses given. Both species form on V8 agar (Wickerham, 1951) club-shaped asci containing a single needle-shaped ascospore and are capable of parasitizing *Daphnia magna* under experimental conditions. *M. zobellii* differs from *M. krissii* by its capacity to ferment glucose and to assimilate galactose, L-sorbose, D-xylose, D-glucosamine, adonitol, D-sorbitol and pyruvate. In infected daphnias the asci and cells of both yeasts resemble Metschnikoff's drawings of *M. bicuspidata*. As cultures of *M. bicuspidata* do not exist and its physiological properties are unknown, the possible identity of either *M. zobellii* or *M. krissii* with *M. bicuspidata* cannot be verified and *M. bicuspidata* (Metschnikoff 1884) Genkel 1913 is therefore considered a *nomen dubium*. Both yeasts were repeatedly isolated from marine substrata on and off the coast of La Jolla, California, U.S.A. Minimum numbers of viable organisms in positive samples varied for *M. zobellii* as follows: sea water, 2–58/100 ml.; fish gut contents (of *Atherinopsis affinis littoralis* and *Trachurus symmetricus*), 25–5,730/ml.; surface of Giant Kelp (*Macrocystis pyrifera*), 520–39,200/g. *M. krissii* was isolated only from sea water, 1–57/100 ml.

INTRODUCTION

Metschnikoff (1884) described as *Monospora bicuspidata* a yeast-like organism, parasitic in the body cavity of the fresh-water crustacean *Daphnia magna*. Keilin (1920) summarized Metschnikoff's observations as follows: 'when the cavity of the hosts is entirely invaded by the parasites, these grow in size, become elongated, and form club- or sausage-shaped asci in each of which is developed a single needle-like spore having both ends pointed. When the parasitized host dies, it is filled with ripe spores, and healthy daphnias, which feed on the detritus of their dead and diseased fellows, become infected by ingesting the asci. The latter, when they enter the host's alimentary canal, set free the needle-shaped spores which perforate the gut wall and penetrate in the body cavity, where they germinate laterally, thus starting the new infection'. It was in this material that Metschnikoff made his early observations on the important phenomenon to which he gave the name of phagocytosis.

According to Kudryavtsev (1954), the generic name *Monospora* was changed by Kamensky (1899) to *Metschnikowia* because *Monospora* had been in use, prior to Metschnikoff, for a genus of Algae. Genkel (1913) found out that *Metschnikowia* had also been in previous use and proposed the name *Metschnikowiella*. Keilin (1920),

unaware of this renaming, introduced the name *Monosporella* and described as *Monosporella unicuspidata* a second species, observed by him in the body cavity of larvae of the peratogonid fly *Dasyhelea obscura*. Kudryavtsev (1954) renamed it *Metschnikowiella unicuspidata*.

Monosporella unicuspidata, as described by Keilin (1920), differs from *Monosporella bicuspidata* by the morphology of its spores: pointed at one end and truncated at the other in the former species, pointed at both ends in the latter. According to Keilin (1920) a similar yeast was observed by Bütschli in the coelom of a free-living nematode, *Tylenchus pellicidus*. Mesnil & Caullery (1911) found yeast-like fungi of elongated shape, in a polychaete worm *Potamilla torelli*, which they considered related to Monospora, although they did not succeed in finding spores. Also in a pelagic copepod *Acartia* they observed what they considered to be a similar yeast. None of these yeasts has been cultivated by their authors or by others. Recently one of us isolated from various marine substrata on and off the coast of La Jolla, California, U.S.A., a number of yeasts, which, based on their fermentative and assimilative properties, belong to two distinct species, both morphologically similar with *Monosporella bicuspidata*. We propose for these two species the names *Metschnikowiella zobellii* and *M. krissii* (in honour of Professor Dr C. E. ZoBell and Professor Dr A. E. Kriss, pioneering marine microbiologists, based at La Jolla, California, U.S.A. and Moscow, U.S.S.R., respectively).

METHODS

Isolation medium. The isolation broth had the following composition (% w/v): glucose, 2; peptone (Difco), 1; yeast extract (Difco), 0.5; filtered sea water; isolation agar had 2% (w/v) agar added. To discourage bacterial growth the medium was adjusted to pH 4.5 with lactic acid.

Isolations from sea water. Subsurface samples were taken at the end of the pier of the Scripps Institution of Oceanography, University of California, La Jolla, California, U.S.A.; 100 ml. samples were run through 'Millipore' filters (Millipore Filter Corp.) of the following specifications: HA, pore size 0.45 μ ; disk diameter 47 mm.; white; plain. Each filter was then placed on top of plated isolation medium.

The plates were incubated at a temperature between 18° and 20°. After 3–5 days yeast colonies appeared on the filters. These were subcultured to the periphery of the plates. The subcultured colonies were distinguished by macroscopic and microscopic morphology. The numbers of each type were recorded and representatives subcultured to slopes of isolation medium for later identification.

Isolations from fish gut contents. Fish of the species *Atherinopsis affinis littoralis* Ayres ('Topsmelt') and *Trachurus symmetricus* Ayres ('Pacific Jack Mackerel') were caught with unbaited hooks off the coast of La Jolla, California. The gut contents of each specimen were suspended in twice their volume of filter-sterilized sea-water and 0.2 ml. amounts of the suspensions were spread with a glass rod on the surface of isolation medium plates. The plates were incubated at 18°–20°, the numbers of yeast colonies recorded according to macroscopic and microscopic morphology. Representative colonies of each type were subcultured for later identification.

Isolations from Giant Kelp. Huge heaps of Giant Kelp (*Macrocystis pyrifera*) are washed ashore on the beaches of Southern California. Pieces of stems and leaves

were cut from the subsurface of such heaps, placed in sterile Erlenmeyer flasks and weighed. After the addition of four times the weight of filter-sterilized sea water, the flasks were shaken for 50 min. Serial dilutions of the wash water were then plated on isolation medium and the plates, treated in the same way as the gut-contents plates.

Identification of the yeast isolates. The methods described by Lodder & Kreger-van Rij (1952), Wickerham (1951) and Van Uden & Farinha (1958), were used. Only isolates belonging to the *Metschnikowiella* genus are considered in this paper.

Experimental pathogenicity. Yeast-free pond water was placed in 50 ml. amounts in wide-mouthed jars together with twenty adult embryo-bearing females of *Daphnia magna* from a yeast-free laboratory population. Duplicate jars were inoculated with a loopful of growth of a 10-day sporulating culture of either *Metschnikowiella zobellii* or *M. krissii*; one set was left uninoculated as a control. The jars were incubated at 20° in a water bath and inspected daily for the presence of dead daphnias. The latter were examined microscopically in unstained preparations and used for making cultures.

RESULTS

A total of twenty-nine isolates of *Metschnikowiella zobellii* and six isolates of *M. krissii* were obtained. Their abundance in the various substrata examined can be seen from Table 1. As the possibility of seasonal fluctuations of the *Metschnikowiella* populations has not been excluded, the dates of collection are given.

Table 1. *Minimum numbers of viable cells of Metschnikowiella spp. yeasts in marine substrata*

Date of collection (1960)	In 100 ml. seawater		<i>M. zobellii</i> in 1 ml. fishgut contents of		<i>M. zobellii</i> on the surface of 1 g. Giant Kelp (<i>Macrocystis pyrifera</i>)
	<i>M. zobellii</i>	<i>M. krissii</i>	<i>Atherinopsis affinis littoralis</i>	<i>Trachurus symmetricus</i>	
12. ii.	8	1	.	.	12,600
19. ii.	28	4	.	.	23,000
21. ii.	2	0	.	.	760
3. iii.	2	0	.	.	5,720
8. iii.	0	14	2,100	0	.
11. iii.	58	0	275	.	.
14. iii.	16	0	.	.	.
15. iii.	0	25	520	0	.
23. iii.	15	30	.	1,950	0
29. iii.	4	0	.	.	.
30. iii.	3	57	.	.	.
1. iv.	.	.	.	5,730	39,200
11. iv.	42	0	0	.	.
9. v.	17	0	85	.	.
10. v.	47	0	930	.	520
11. v.	22	0	.	25	29,600
13. v.	15	0	.	.	0

0 = no yeast present in sample.

. = no sample taken.

Metschnikowiella zobellii sp.nov.

In medio liquido cum dextroso et peptono et extracto levedinis cellulae rotundae et ovoideae, $(6-11) \times (7-11) \mu$, singulares, binae aut catenatae; pellicula tenuis formatur. In agaro peptonato cum dextroso et extracto levedinis cultura flavalbida, mollis, subnitida, subreticulata. Pseudomycelium primitivum formatur. Asci in agaro V8 clavati, $18-20 \mu$; asci in *Daphnia magna* elongato-subclavati, $30-45 \mu$. Ascosporae acuiiformes, bicuspidatae, ad 1 in asco; in agaro V8, $15-18 \mu$, in *Daphnia magna*, $28-43 \mu$. Dextrosum fermentatur at non galactosum, saccharum, maltosum, raffinose, trehalosum et inulinum. Dextrosum, galactosum, L-sorbose, maltosum, saccharum, cellobiosum, trehalosum, meleucitosum, D-xylosum, D-glucosaminum, alcohol aethylicum, glycerinum, adonitolum, D-mannitolum, D-sorbitolum, alpha-methyl-glucosidum, salicinum, arbutinum, acidum pyruvicum et acidum succinicum assimilantur at non nitrates calicis. Necessaria ad fortem crescentiam sunt vitamina externa: biotinum et thiaminum. Temperatura maxima crescentiae $34^{\circ}-35^{\circ}$.

Morphology. In isolation broth after 48 hr. at 25° cells are round and oval, $(6-11) \times (7-11) \mu$, single, in pairs and in small groups. A thin pellicle is formed. The streak culture on isolation agar after 30 days at 25° is yellowish white, soft, dull-glistening and slightly reticulated. A primitive pseudomycelium is formed.

Sporulation. Asci are formed on Henrici's vegetable juice medium (V8 medium, Wickerham, 1951). No sporulation has been observed to occur on isolation agar, malt agar and corn-meal agar. The asci are club-shaped, $18-21 \mu$ long and contain a single, needle-shaped ascospore, pointed at both ends and $15-18 \mu$ long (Fig. 1).

Fermentation, growth in the absence of single vitamins at 25° and assimilation (see Table 2).

Maximum temperature still permitting growth: $34^{\circ}-35^{\circ}$.

Experimental pathogenicity for Daphnia magna. During the 21 days of observation daphnia populations in the control jars showed no signs of disease and produced a numerous offspring. In the jars inoculated with *Metschnikowiella zobellii*, dead daphnias began to appear after 3-5 days and the entire population had died after 10-14 days. The dead specimens were densely filled with yeasts; on subculture the dead daphnias produced only *M. zobellii*, no other yeasts. The morphology of *M. zobellii* in the daphnias showed a striking similarity with Metschnikoff's drawings of *M. bicuspidata*, as reproduced by Keilin (1920) and Kudryavtsev (1954). The asci were elongated or slightly clavate, $30-45 \mu$ long, and contained a single needle-shaped ascospore, $28-43 \mu$ long. The vegetative cells were more slender than the cells on artificial media (Fig. 2).

Type strain. An isolate from sea water has been designated as the type strain of *Metschnikowiella zobellii*. It is maintained in this laboratory with the number 2892. Subcultures have been deposited with the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, Netherlands and the Fermentation Division of the Northern Utilization Research and Development Division, Peoria, Illinois, U.S.A.

Metschnikowiella krissii sp.nov.

In medio liquido cum dextroso et peptono et extracto levedinis cellulae rotundae et ovoideae, $(4.5-6) \times (6-11) \mu$ et longovoideae, $(4.5-6) \times (11-13) \mu$, singulares, binae

aut catenatae. In agar peptonato cum dextroso et extracto levedinis cultura flavalbida, mollis, subnitida, subpunctata. Pseudomycelium primitivum formatur. Asci in agar V8 clavati, 18–24 μ ; asci in *Daphnia magna* elongato-subclavati vel elongato-angulares, 35–40 μ . Ascospores acuiiformes, bicuspidatae, ad 1 in asco; in agar V8, 15–21 μ ; in *Daphnia magna* 32–38 μ . Non fermentat. Dextrosus, maltosus, saccharus, cellobiosus, trehalosus, meleucosus, alcohol aethylicus, glycerinus, D-mannitolus, alpha-methyl-glucosidus, salicinus, arbutinus et acidum succinicum assimilantur, ad non nitras kalicus. Necessaria ad fortem crescentiam sunt vitamina externa: biotinus et thiaminus. Temperatura maxima crescentiae 34–35°.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1. *Metschnikowiella zobellii*. Asci and vegetative cells on V8 medium. Camera lucida drawing.

Fig. 2. *Metschnikowiella zobellii*. Asci and vegetative cells in parasitized *Daphnia magna*. Camera lucida drawing.

Fig. 3. *Metschnikowiella krissii*. Asci and vegetative cells on V8 medium. Camera lucida drawing.

Fig. 4. *Metschnikowiella krissii*. Asci and vegetative cells in parasitized *Daphnia magna*. Camera lucida drawing.

Morphology. In isolation broth after 48 hr. at 25° cells are round and oval, (4.5–6) × (6–11) μ , and long oval, (4.5–6) × (11–13) μ , single, in pairs and small groups. A thin pellicle may form after prolonged incubation. The streak culture on

Table 2. *Fermentation, vitamin dependency and assimilation patterns of Metschnikowiella zobellii*

Fermentation + gas - gas	Growth in the absence of single vitamins at 25°		Assimilation	
	Without biotin ±	Glucose +	Raffinose -	d-Glucosamine +
Glucose	Without pantothenate +	Galactose +	Melecitose +	Salicin +
Galactose	Without inositol +	L-Sorbose +	Inulin -	Arbutin +
Sucrose	Without niacin +	Maltose +	Soluble starch -	Pyruvate +
Maltose	Without folic acid +	Sucrose +	d-Xylose +	Lactate -
Lactose	Without <i>p</i> -aminobenzoic acid +	Cellobiose +	L-Arabinose -	Succinate +
Raffinose	Without pyridoxin +	Trehalose +	d-Arabinose -	Citrate -
Trehalose	Without riboflavin +	Lactose -	d-Ribose -	Ethylacetate -
Inulin	Without thiamin ±	Melibiose -	L-Rhamnose -	Inositol -
			+ = good growth, ± = weak growth, - = no growth.	

Table 3. *Fermentation, vitamin dependency and assimilation patterns of Metschnikowiella krissii*

Fermentation - gas	Growth in the absence of single vitamins at 25°		Assimilation	
	Without biotin ±	Glucose +	Raffinose -	d-Glucosamine -
Glucose	Without pantothenate +	Galactose -	Melecitose +	Salicin +
Galactose	Without inositol +	L-Sorbose -	Inulin -	Arbutin +
Sucrose	Without niacin +	Maltose +	Soluble starch -	Pyruvate -
Maltose	Without folic acid +	Sucrose +	d-Xylose -	Lactate -
Lactose	Without <i>p</i> -aminobenzoic acid +	Cellobiose +	L-Arabinose -	Succinate +
Raffinose	Without pyridoxin +	Trehalose +	d-Arabinose -	Citrate -
Trehalose	Without riboflavin +	Lactose -	d-Ribose -	Ethylacetate -
Inulin	Without thiamin ±	Melibiose -	L-Rhamnose -	Inositol -
			+ = good growth, ± = weak growth, - = no growth.	

isolation agar after 30 days at 25° is yellowish white, soft, dull-glistening and slightly pointed. A primitive pseudomycelium is formed.

Sporulation. Asci are formed abundantly on V8 medium (Wickerham, 1951). Sporulation has not been observed on isolation agar, malt agar and corn meal agar. The asci are club-shaped, 18–24 μ long and contain a single, needle-shaped ascospore, pointed, at both ends and 15–21 μ long (Fig. 3).

Fermentation, growth in the absence of single vitamins at 25° and assimilation (see Table 3).

Maximum temperature still permitting growth: 34°–35°.

Experimental pathogenicity for Daphnia magna. *Metschnikowiella krissii* behaved in the same way as *M. zobellii*. Its morphology in *Daphnia magna* can be seen in Fig. 4. Many asci were slightly angular.

Type strain. An isolate from sea water has been designated as the type strain of *M. krissii*. It is maintained in this laboratory with number 2895. Subcultures have been deposited with the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, Netherlands, and the Fermentation Division of the Northern Utilization Research and Development Division, Peoria, Illinois, U.S.A.

DISCUSSION

Taxonomy. As *Metschnikowiella bicuspidata* and *M. unicuspidata* have not been cultivated in the laboratory, their physiological properties are unknown and no cultures are available for comparative studies. As yeast species identification is largely based on physiological properties, an eventual re-identification of either species would probably imply an arbitrary decision. Their known properties, as described by Metschnikoff and Keilin, are however unique among the yeasts and characterize the genus *Metschnikowiella*: (1) asci containing a single needle-shaped ascospore; (2) pathogenicity for arthropods. We feel justified therefore to place *M. zobellii* and *M. krissii* in *Metschnikowiella*. An emended description of the genus *Metschnikowiella*, a discussion of its possible phylogenetic relationships and the designation of a type species is better postponed, we feel, till the life cycles of *M. zobellii* and *M. krissii* have been worked out. Both *M. zobellii* and *M. krissii* are morphologically similar to *M. bicuspidata* and both are capable, like *M. bicuspidata*, of parasitizing *Daphnia magna*. As far as Metschnikoff's description of *M. bicuspidata* goes, our two yeasts would have to be identified with Metschnikoff's organism. This, however, is impossible since *M. zobellii* and *M. krissii* belong to distinct species. *M. zobellii* differs from *M. krissii* by its capacity to ferment glucose and to use galactose, L-sorbose, D-xylose, D-glucosamine, adonitol, D-sorbitol, and pyruvate. This situation exemplifies that Metschnikoff's description of *M. bicuspidata* is not sufficiently complete to allow its re-identification. As, furthermore, no cultures of *M. bicuspidata* are available to permit an emended description, we have to consider the name *Metschnikowiella bicuspidata* (Metschnikoff, 1884) Genkel, 1913 as a *nomen dubium*.

Ecology. *Metschnikowiella zobellii* and *M. krissii* were repeatedly isolated from sea water and *M. zobellii* also from Giant Kelp and fish gut (Table 1). This shows that both species, though capable of infecting and killing *Daphnia magna*, are facultative, rather than obligate parasites. The numbers of viable *M. zobellii* on the surface of Giant Kelp were very high as compared with the numbers found in sea water. In

most heaps of Giant Kelp from which *M. zobellii* was isolated, slight signs (smell, consistency, temperature) of beginning microbial decomposition were noted. Possibly the breakdown of algal polysaccharides by bacterial action provides certain simple carbohydrates, easily used as a carbon source by *M. zobellii*. The inability of *M. krissii* to assimilate galactose and other carbohydrates may possibly explain why this species was not found in the kelp heaps. The numbers of *M. zobellii* were much higher in fish gut than in sea water. This suggests that *M. zobellii* either multiplies in fish gut more actively than in sea water or is associated with some of the marine organisms on which the fish feed. Both *M. zobellii* and *M. krissii* are capable of parasitizing *Daphnia magna* under experimental conditions. *Daphnia magna*, however, is a fresh-water crustacean, whereas *M. zobellii* and *M. krissii* seem well adapted to marine environments. Whether these species may, under natural or experimental conditions, parasitize marine crustaceans or other marine animals is an open question.

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REFERENCES

- GENKEL, A. (1913). *Essay on Sporulating Plants*. Moscow: Knebel.
- KAMENSKY, F. M. (1899). On a new view of the genus *Metschnikowia* (Monospora Metschnikoff). *Treat. spb. soc. natural.* (Russian), **30**, part 5.
- KEILIN, D. (1920). On a new saccharomycete *Monosporella unicuspidata* gen. n.nom., n.sp., parasitic in the body cavity of a dipterous larva (*Dasyhelea obscura* (Winnertz). *Parasitology*, **12**, 83.
- KUDRYAVTSEV, V. I. (1954). *Systematics of the Yeasts*. Moscow: Ysdatelstwo Akademii Nauk SSSR.
- LODDER, J. & KREGER-VAN RIJ, N. J. W. (1952). *The Yeasts*. Amsterdam: North Holland Publishing Company.
- MESNIL, F. & CAULLERY, M. (1911). Neoformations papillomateuses chez une Annélide (*Potamilla torellii*) dues probablement à l'influence de parasite (Haplosporidie et levure). *Bull. Sci. Fr. Belg.* **45**, 89.
- METSCHNIKOFF, E. (1884). Über eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre über den Kampf der Phagocyten gegen Krankheitserreger. *Virchows Arch.* **96**, 177.
- VAN UDEN, N. & FARINHA, M. (1958). On the significance of temperature relations and vitamin deficiency patterns in the delimitation of yeast species. *Port. Acta biol.* (B), **6**, 161.
- WICKERHAM, L. J. (1951). Taxonomy of Yeasts. *Tech. Bull. U.S. Dep. Agric.* no. 1029.