Short Communication

METHOD FOR ISOLATION OF MATING-LESS MUTANTS FROM *RHODOSPORIDIUM TORULOIDES* M 1057, A STRAIN OF MATING TYPE *a*

KEIKO ABE, EIKO TSUCHIYA, SAKUZO FUKUI,
NORIKO KODAMA,* AND AKIO KUSANAGI*

Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo

**Faculty of Science, Yokohama City University, Kanagawa-ku, Yokohama, Japan*

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Rhodosporidium toruloides, a heterobasidiomycetous yeast which is bipolar in sexual compatibility, shows a peculiar change in cell morphology during the early stages of sexual reproduction (1–3). The haploid cell of the microorganism carries the gene of mating type *A* or *a*, and propagates as yeast by budding. However, when the compatible mating-typed cells are mixed, the induction of mating tube formation occurs in both *A*- and *a*-typed cells (*A* and *a* cells), followed by elongation of the tubes. Then, cell fusion takes place between *A* and *a* cells at the apex of the tube. After the cell fusion, secondary hypha formation occurs in the plasogamete cells, and the hypha grows as dikaryotic cells with clamp connections. In the previous papers, we reported that *A* and *a* cells produce extracellular diffusible substances inducing the formation of mating tubes in the opposite mating-typed cells, *a* and *A* cells, respectively (3, 4). Recently, rhodotorucine *A* was isolated from the culture fluid of *A* cells (5, 6), and its chemical structure was confirmed to be (H) Tyr-Pro-Glu-Ile-Ser-Trp-Thr-Arg-Asn-Gly-Cys-S-farnesyl ether (in press in B.B.R.C.). A concentration of 8 ng/ml of rhodotorucine *A* is sufficient to induce sexual differentiation in *a* cells, in which both bud formation and nucleus duplication are repressed and mating tube formation is induced as a biological response to the sex hormone (7).

In this paper, we describe a method for isolation of mating-less mutants, which are considered to be the effective tools to obtain biological and biochemical solution for the mechanism of sexual reproduction, from *Rhodosporidium toruloides* M1057, a strain of mating type *a*, and some properties of the mutants isolated are also presented.

Rhodosporidium toruloides IFO 0559–M919 (haploid yeast, mating type *A*, *Met*[–], *Pan*[–]) and IFO 0880–M1057 (haploid yeast, mating type *a*, *Paba*[–]) were

designated as M919 (or *A* cell) and M1057 (or *a* cell), respectively, and used in this paper. Abbreviations were described in our previous paper (3). YS liquid medium (YS), which contained 10 g of yeast extract (Daigo-Eiyo, Osaka), 10 g of sucrose, and 2.5 g of NaCl per liter of deionized water, was used for cultivation of haploid strains. Minimal agar medium (MM), which contained 30 g of sucrose, 3 g of $(\text{NH}_4)_2\text{SO}_4$, 1 g of KH_2PO_4 , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g of NaCl, and 15 g of agar per liter of deionized water (pH 6.5), was used as selection medium for mated cells. For determination of mating ability of *a* cells, PABA-MM, which contained 0.2 mg of *p*-aminobenzoic acid per liter of MM, was used. Mating ability, a parameter of sexual reproduction, was determined as described below. Both *A* and *a* cells were separately precultured in YS (5 ml) in a test tube (16 $\times$ 180 mm) with a reciprocal shaking (100 strokes/min) at 25°. Early log-phase cells of M1057 (10^7 cells/ml) were diluted to a cell concentration of approximately 10^3 /ml with a sterilized saline. Early log-phase cells of M919 (10^7 cells/ml) were collected by centrifugation and resuspended in a sterilized saline to give a cell concentration of 10^7 /ml. Then, 0.1 ml each of these cell suspensions were mixed on PABA-MM plate (cell number ratio of *a* and *A* cells, 1: 10^4), spread well, and incubated at 25° and 33° for 5 days. On the plate, mated and non-mated cells of M1057 gave brown hyphal-colony and yellow round-one, respectively, while M919 did not give colony. Mating ability is presented as a percentage of colony number of mated cells to total number of colonies.

The ability of mating tube formation, which is a parameter of induction of sexual differentiation as a response to rhodotorucine *A*, was determined as described below. Approximately 10^8 cells of M1057 (early log-phase) were adhered on a cover glass according to the method reported previously (4), and the glass was placed in 10 ml of the culture supernatant of M919 containing 60 units of rhodotorucine *A* per ml, followed by incubation at both 25 and 33° for 5 hr. The number of mating tube-formed cells was counted under a microscope. The ability of mating tube formation was presented as a percentage of mating tube-formed cells per total cells. One unit of the hormone was the amount which shows a mating tube-forming ability of 30% at 25°.

Mating-less mutants of *a* strain was isolated as described below. An amount of 5 ml of the cell suspension (10^7 cells/ml) of M1057 (early log-growth phase) in saline was placed in a sterilized petri dish, and irradiated with UV ray for 3 min with gentle stirring (survival, approximately 5%). Then, segregation culture was performed in YS at 25° for 15 hr. Segregants were plated on YS agar and incubated at 25° for 5 days. The colonies thus formed were replicated on MM, on which 10^6 cells of M919 per plate were previously spread (just before replication), followed by incubation at 25° for 5 days. The colonies which did not give brown hyphal-colonies on MM were isolated as mating-less mutants.

Ninety-seven strains of mating-less mutants were isolated and grouped by two phenotypical parameters, such as mating tube-forming ability and mating

Table 1. Groupings of mating-less mutants.

Parameters	25°		33°		Mutant strains
	Mating tube-forming ability	Mating ability	Mating tube-forming ability	Mating ability	
Group I	+	+	+	—	2, 3, 9, 17, 18, 35, 59, 62, 63
Group II	+	+	—	—	None
Group III	+	—	+	—	4, 7, 8, 11, 12, 21, 24, 25, 28, 29, 36, 37, 43, 50, 51, 52, 53, 57, 61
Group IV	+	—	—	—	None
Group V	—	—	—	—	20, 31, 38, 39, 40, 47, 60
Wild type	+	+	+	+	M1057

+, Ability is higher than one-tenth of that measured with wild-type strain; —, ability is less than one-hundredth of that measured with wild-type strain.

Table 2. Phenotypes of typical strains of mating-less mutants.

	25°		33°	
	Mating tube-forming ability (%)	Mating ability (%)	Mating tube-forming ability (%)	Mating ability (%)
Mutant-2 (Group I)	> 70	25	> 70	0
Mutant-21 (Group III)	30–70	0	30–70	0
Mutant-39 (Group V)	0	0	0	0
Mutant-34 (Intermediary group)	10–30	3.4	10–30	1.6
M1057 (Wild type)	> 70	80	> 70	45

ability. Groupings in Table 1 also include thermosensitivity of the parameters; 25° and 33° are permissive and non-permissive temperatures, respectively. Nine strains in the mutants were thermosensitive in mating ability (Group I), nineteen strains were negative in mating ability and positive in mating tube-forming ability at both 25° and 33° (Group III), and seven strains were negative in mating tube-forming ability (Group V). The strains belonging to Groups II and IV, which are thermosensitive in the mating tube-forming ability, could not be isolated within the present work. The remaining sixty-seven strains showed intermediary properties in the parameters. Typical strains of the mutants are summarized in Table 2.

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