Rhodotorula cresolica sp. nov., a Cresol-Assimilating Yeast Species Isolated from Soil

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A cresol-assimilating yeast strain of a previously undescribed species belonging to the genus *Rhodotorula* was isolated from soil. The new strain differs from the previously described species of the genus in its pattern of assimilation of carbon and nitrogen compounds, G+C content, and low levels of DNA-DNA-homology. The new species *Rhodotorula cresolica* is described. The type strain is CBS 7998.

Several yeast species are able to assimilate a great variety of benzene compounds as sole sources of carbon and energy. Strains displaying this property have been found in culture collections (5) and in several natural habitats (7, 12). In a previous publication, the isolation of yeast strain G36^T (T = type strain) from soil polluted with *ortho*-cresol was reported (7). Inoculation with strain G36^T of soil contaminated with *o*-cresol accelerated the degradation of this pollutant (7), thus demonstrating that strain G36^T can successfully compete for *ortho*-cresol with the general soil flora. Identification of this organism was based on physiological properties (i.e., the pattern of assimilation of carbon and nitrogen compounds). The results revealed similarity between strain G36^T and *Rhodotorula aurantiaca* (Saito) Lodder. However, strain G36^T lacked the red pigment and the tendency to elongate to cylindrical cells characteristic of *R. aurantiaca*. Moreover, it grew without vitamins. This prompted us to reconsider the taxonomic position of strain G36^T. The results of DNA base composition and DNA-DNA reassociation studies led us to propose a novel species, named *Rhodotorula cresolica* Middelhoven et Spaaij. Strain G36 (= CBS 7998) is the type strain of this new taxon.

MATERIALS AND METHODS

Isolation of the strain. Acidic sandy soil (pH [H₂O] 3.9; moisture content, 10%) obtained just beneath the litter layer of a 130-year-old oak grove at Wageningen, The Netherlands, was supplied with *ortho*-cresol (about 20 mg/500 g of soil). The contaminated soil was incubated at 10°C in a covered beaker for 2 months. From time to time the contents were mixed with a sterile spatula. Yeasts were enriched by shaking a slurry containing 2.5 g of polluted soil with 10 ml of 1% glucose–1% yeast extract for 2 days at 20°C. This culture was used as an inoculum for slant cultures (6) containing *o*-cresol as the sole carbon source. Strain G36^T was obtained by streaking material from the zone of growth on YM agar. The strain was maintained in the same medium.

Characterization of the strain. Strain G36^T was examined for its morphological, cultural, and physiological properties by using the standard methods adopted for yeast identification (15). Utilization of carbon and nitrogen sources was examined at 25°C on a rotary shaker rotating at a speed of 100 rpm. Utilization of nitrogen sources was confirmed by the auxanographic technique. Assimilation of potentially toxic benzene compounds was studied by the slant culture method (6).

To determine the DNA base composition, cells were grown in 2% GYP (15) at 25°C on a rotary shaker until the late log phase. Cells were harvested by centrifugation, washed in water and saline EDTA (0.1 M NaCl, 0.15 M EDTA), and broken with a French press. DNA was isolated by the method of Cryer et al. (2) and was purified by centrifugation in a cesium chloride-ethidium bromide gradient as described by Maniatis et al. (4). The purity of the DNA preparation was checked by the spectrophotometric method (4). The base composition of the DNA was determined in 0.1× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0) from the thermal denaturation profile (9) with a

Gilford Response UV-VIS spectrophotometer and thermal programming software heating at a rate of 0.1°C/min. The G+C content was calculated by the following formula: G+C content = $(2.08 \times T_m) - 106.4$, where T_m is the thermal denaturation temperature. A standard preparation of *Candida parapsilosis* CBS 604 DNA (T_m , 70.6°C) was included as a control in every determination. The value reported below is the mean ± standard deviation from three determinations. The extent of DNA-DNA reassociation was determined by using the same instrument (3, 13).

Ubiquinone isoprenologs were extracted and purified as described previously (16, 17). To determine the type of coenzyme Q (CoQ), we used reverse-phase thin-layer chromatography, Merck HPTLC RP-18 F254s plates, and acetone-acetonitrile (80:20, vol/vol) as the developing solvent (8). The separated components were detected under UV light at 254 nm.

RESULTS

Latin diagnosis of *Rhodotorula cresolica* Middelhoven et Spaaij sp. nov. In medio liquido dextrosus et peptonum et extractum levidinis continenti post 3 dies ad 20°C cellulae sunt globosae aut ovoideae (2–11 × 2–9 µm), singulae aut binae. Post hebdomades 4 sedimentum adest. Pelliculum non formatur. Cultura in agar dextrosus et peptonum et extractum levidinis post hebdomades 4, 20°C, viscosa, crema, glabra, nitida, margo integer. In agar farina *Zea maydis* confecto post dies 7, 20°C nec mycelium nec pseudomycelium formantur. Fermentatio nulla. Assimilat D-glucosum, D-glucosaminum (lente), D-ribosum (lente), D-xylosum, D-arabinosum (lente), sucrosus, maltosus, trehalosus, α-methyl-D-glucosidus, cellobiosus (lente), arbutinus, melezitosis, glycerolus, ribitolus, xylitolus, glucitolus, mannitolus, glucono-δ-lactonus, acidum 2-ceto-D-gluconicum, acidum 5-ceto-D-gluconicum, acidum gluconicum, acidum glucuronicum, acidum galacturonicum, acidum lacticum (lente), acidum succinicum, acidum citricum (lente), ethanolus, propano-1,2-diolus, acidum quinicum, acidum saccharicum (lente). Non assimilat D-galactosum, L-sorbosum, L-arabinosum, L-rhamnosum, salicinum, melibiosum, lactosus, raffinosis, inulinus, amyllum solubile, erythritolus, L-arabinitolus, galactitolus, inositolus, methanolus, butano-2,3-diolus. Assimilat kalii nitratus, sodii nitratus, ethylaminus, L-lysinus, cadaverinus, creatinus, creatininus. Non assimilat glucosaminus et imidazolus.

Vitamina externa ad crescentiam non necessariae sunt. Crescit in 25°C, non crescit in 30°C. Materiae amyloideae non formantur. Ureum finditur. Proportio guanini plus cytosini in acido deoxyribonucleico 49.2 ± 0.4 per centum.

Typus: CBS 7998 isolatus ex terra Wageningen in collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum.

Morphological characteristics of the species. After 5 days of growth in broth containing glucose (2%, wt/vol), yeast extract (0.5%, wt/vol), and peptone (1%, wt/vol) at 20°C, the cells are globose to ovoid (2 to 11 by 2 to 9 µm). A pellicle is not

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TABLE 1. Assimilation of carbon compounds

Compound	Assimilation by strain G36 ^T
D-Glucose	+ ^a
D-Galactose	—
L-Sorbose	—
D-Glucosamine	D
D-Ribose	D
D-Xylose	+
L-Arabinose	—
D-Arabinose	D
L-Rhamnose	—
Sucrose	+
Maltose	+
αα-Trehalose	+
Methyl-α-D-glucoside	+
Cellobiose	D
Salicin	—
Arbutin	+
Melibiose	—
Lactose	—
Raffinose	—
Melezitose	+
Inulin	—
Starch	—
Glycerol	+
Erythritol	—
Ribitol	+
Xylitol	+
L-Arabinitol	—
D-Glucitol	+
D-Mannitol	+
Galactitol	—
myo-Inositol	—
D-Glucono-1,5-lactone	+
2-Keto-D-gluconate	+
5-Keto-D-gluconate	+
D-Gluconate	+
D-Glucuronate	+
D-Galacturonate	+
DL-Lactate	D
Succinate	+
Citrate	D
Methanol	—
Ethanol	+
Propane-1,2-diol	+
Butane-2,3-diol	—
Quinate	+
Saccharate	D
Galactonate	D, W
n-Hexadecane	—

^a +, positive; —, negative; D, delayed; W, weak.

formed, but a sediment is present. A streak culture in this medium solidified with agar (2%) after 4 weeks at 20°C is cream colored, mucoid, glistening, and smooth with entire margins. No mycelium or pseudomycelium is formed in a slide culture in corn meal agar after 5 days at 20°C.

Alcohol fermentation. No detectable gas is formed during alcohol fermentation.

Assimilation of carbon and nitrogen compounds. The assimilation of carbon compounds and the assimilation of nitrogen compounds are shown in Tables 1 and 2, respectively. The assimilation of benzene compounds is shown in Table 3.

Other characteristics. External vitamins are not required. Grows at 25°C, but not at 30°C. No growth occurs in 50% glucose-yeast extract agar and in the presence of 0.01 or 0.1% cycloheximide. Amyloid compounds are not produced. Urea is

hydrolyzed. The color reaction with Diazonium Blue B is positive. The G+C content of the nuclear DNA is 49.2 ± 0.4 mol%. The major CoQ is CoQ 10.

Etymology. The specific epithet *cresolica* pertains to cresols; *cresolica*, referring to the ability to assimilate cresols.

Origin and deposits. Strain G36^T was isolated from soil in Wageningen, The Netherlands. Type strain CBS 7998 has been deposited in the yeast culture collection of the Centraalbureau voor Schimmel-cultures in Delft, The Netherlands, and in the culture collection of the Laboratory of Microbiology in Wageningen, The Netherlands.

DISCUSSION

Besides the differences in utilization of carbon and nitrogen compounds (Table 4), our isolate lacks the red pigment, the tendency to elongate to cylindrical cells, and the thiamine requirement characteristic of strains belonging to *R. aurantiaca*. The type strain of *R. aurantiaca*, ATCC 32770 (= CBS 317), also does not assimilate phenol, the cresols, hydroquinone, and resorcinol, but grows slowly on 4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid (data not shown). The two taxa are genetically distinct and represent separate anamorphic species. This conclusion was based on the G+C values, which differed by more than 2 mol%, and on the low level of DNA-DNA reassociation. The levels of reassociation between *R. cresolica* DNA and DNAs from *R. cresolica* CBS 7998^T, *R. aurantiaca* CBS 317, and *Rhodotorula pilatii* CBS 7039^T were 98, 0, and 20%, respectively (in all cases the standard deviation was less than 5%).

Identification of strain G36^T with the determination keys provided by Barnett et al. (1) also pointed to *Rhodotorula pilatii* (Jacob et al.) Weijman (Barnett et al. 1983). The type strain of this species, CBS 7039, slowly assimilated phenol, hydroquinone, and 3-hydroxybenzoic acid (data not shown). Other strains of this species have been isolated from enrichment cultures inoculated with wood or shelf fungi by using protocatechuic acid or vanillic acid as the sole energy source (12). The characteristics which differentiate the species are listed in Table 4. Again, lack of DNA-DNA reassociation excluded placement of strain G36^T in this species (see above).

The pattern of assimilation of carbon and nitrogen compounds of strain G36^T is very similar to that of *Leucosporidium scottii* Fell et al. Nonmycelial strains of this species are sometimes isolated from soil and other habitats. *L. scottii* is known to assimilate many benzene compounds (5), including *meta*- and *para*-cresol, but not *ortho*-cresol. Strain G36^T cannot be identified as *L. scottii* as the G+C content of strain G36^T is 49.2 ± 0.4 mol% and the G + C content of *L. scottii* 59.0 mol%

TABLE 2. Assimilation of nitrogenous compounds

Compound	Assimilation by strain G36 ^T
Nitrate	+ ^a
Nitrite	+
Ethylamine	+
L-Lysine	+
Cadaverine	+
Creatine	+
Creatinine	+
Glucosamine	—
Imidazole	—

^a +, positive; —, negative.

TABLE 3. Assimilation of benzene compounds as determined by the slant culture method

Compound ^a	Assimilation by strain G36 ^T
Phenol (hydroxybenzene).....	+ ^b
Anisole (methoxybenzene).....	—
Catechol (1,2-dihydroxy).....	+
Resorcinol (1,3-dihydroxy).....	+
Hydroquinone (1,4-dihydroxy).....	+
Phloroglucinol (1,3,5-trihydroxy).....	—
Pyrogallol (1,2,3-trihydroxy).....	—
ortho-Cresol (2-methylphenol).....	D
meta-Cresol (3-methylphenol).....	+
para-Cresol (4-methylphenol).....	+
3-Methylcatechol (1,2-dihydroxy-3-methylbenzene).....	—
4-Methylcatechol (1,2-dihydroxy-4-methylbenzene).....	+
Orcinol (1-methyl-3,5-dihydroxybenzene).....	+
Benzyl alcohol (hydroxymethylbenzene).....	+
Benzoic acid.....	+
Salicylic acid (2-hydroxy).....	—
3-Hydroxybenzoic acid.....	—
4-Hydroxybenzoic acid.....	+
2,3-Dihydroxybenzoic acid.....	+
2,4-Dihydroxybenzoic acid.....	+
Gentisic acid (2,5-dihydroxy).....	—
Protocatechuic acid (3,4-dihydroxy).....	+
3,5-Dihydroxybenzoic acid.....	+
2,3,4-Trihydroxybenzoic acid.....	—
Gallic acid (3,4,5-trihydroxy).....	—
Vanillic acid (3-methoxy-4-hydroxy).....	+
Isovanillic acid (3-hydroxy-4-methoxy).....	+
Veratric acid (3,4-dimethoxy).....	+
Syringic acid (4-hydroxy-3,5-dimethoxy).....	—
4-Ethylphenol.....	—
1-Phenylethanol.....	—
2-Phenylethanol.....	—
2-Phenylethylamine (phenethylamine).....	+
Tyramine (4-hydroxyphenylethylamine).....	+
Phenethylene glycol (2-phenylethane-1,2-diol).....	+
Acetophenone (acetylbenzene).....	—
4-Hydroxyacetophenone.....	+
4-Methoxyacetophenone.....	—
Phenylacetic acid.....	+
2-Hydroxyphenylacetic acid.....	—
3-Hydroxyphenylacetic acid.....	W
4-Hydroxyphenylacetic acid.....	+
Homoprotocatechuic acid (3,4-dihydroxy).....	W
Homogentisic acid (2,5-dihydroxy).....	—
DL-Mandelic acid (2-phenyl-2-hydroxyacetic acid).....	+
DL-4-Hydroxymandelic acid.....	+
3-Phenylpropionic acid.....	+
L-3-Phenyllactic acid.....	—
DL-4-Hydroxyphenyllactic acid.....	—
Phenylpyruvic acid.....	—
L-Phenylalanine.....	+
L-Tyrosine.....	—
trans-Anethole (4-propenylanisole).....	—
Cinnamic acid (trans-3-phenylpropenoic acid).....	+
3-Hydroxycinnamic acid.....	—
4-Hydroxycinnamic acid (4-coumaric acid).....	+
Coumarin.....	D, W
Caffeic acid (3,4-dihydroxycinnamic acid).....	+
Ferulic acid (3-methoxy-4-hydroxy).....	+
Sinapinic acid (4-hydroxy-3,5-dihydroxy).....	—
4-Phenylbutyric acid.....	D
5-Phenylvaleric acid.....	+
1-Phenyl-n-dodecane.....	—

^a Benzene compounds are listed in order of side chain length.^b +, positive; —, negative; D, delayed; W, weak.TABLE 4. Characteristics that differentiate *R. cresolica*, *R. aurantiaca*, and *R. pilatii*

Compound	Utilization by:		
	<i>R. cresolica</i>	<i>R. aurantiaca</i>	<i>R. pilatii</i>
L-Sorbose	— ^a	—, D	+, D
D-Glucosamine	+, D	—	—
D-Ribose	+, D	—, D	—
α-Methyl-D-glucoside	+	—	—
Lactose	—	—	D
DL-Lactate	+, D	—	+, D
L-Lysine	+	+, —	—
Cadaverine	+	—	—
Creatine	+	—	—
Creatinine	+	—	—

^a —, negative; +, positive; D, delayed.

(14). Moreover, the major CoQs of *L. scottii* are CoQs 9 and 10 (14).

The lack of DNA similarity with any of the above-mentioned physiologically related species made defining a new taxon to accommodate our isolate unavoidable. The novel species is placed in the imperfect basidiomycetous genus *Rhodotorula* Harrison as strain G36^T fails to produce amyloid compounds and to grow at the expense of *myo*-inositol. In addition to cresols, the strain is able to assimilate numerous benzene compounds (Table 3). In this respect this organism resembles other basidiomycetous yeasts, such as *Rhodotorula bogoriensis* (Deinema) Von Arx et Weijman CBS 4101, *Rhodotorula glutinis* (Fresenius) Harrison St. 41, *L. scottii* G43, and various *Trichosporon* species (5). However, the ability to assimilate *ortho*-cresol is unique among the basidiomycetous yeasts tested (5). In a yeast collection comprised of 29 different species that were screened for assimilation of 84 different benzene compounds, only the black ascomycetous yeastlike fungus *Exophiala jeanselmei* (Langer.) McGinnis et Padhye was able to assimilate *ortho*-cresol (5). No data on the biodegradative pathway for *ortho*-cresol in yeasts are available. The degradative pathway for *para*-cresol in *Trichosporon cutaneum* has been elucidated (10, 11). Like all other yeasts tested previously, *R. cresolica* G36^T failed to grow on benzene compounds with more than one carbon side chain (e.g., xlenols and toluic and phthalic acids) or a branched carbon side chain (5).

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