Microbiol. Cult. Coll. Dec. 1997. p. 109-112 Vol. 13, No. 2

Candida gotoi, New Species of Anamorphic Yeast Isolated from Insect Frass in Bark of Japanese Maple

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A strain of yeast isolated from insect frass of a Japanese maple (*Acer palmatum* Thunberg) in 1982 was found to represent a new species of anamorphic yeast and was described as *Candida gotoi* Nakase et Suzuki. This yeast was characterized by Q-8 as the major ubiquinone, glucan-mannan type cell wall, and mol% G+C of DNA of 41.6. Among species with Q-8, *C. gotoi* resembles *C. silvanorum* in the mol% G+C and in taxonomic criteria commonly employed but is differentiated from it by a low DNA homology value of 19%. Practically, *C. gotoi* is distinguished from *C. silvanorum* by its ability to assimilate galactitol and 2-ketogluconic acid, and inability to assimilate melibiose.

Key words : new yeast, *Candida gotoi*

INTRODUCTION

In the course of a survey of yeasts associated with insect frass in bark, one of the authors (T.N) isolated an anamorphic yeast strain with Q-8 as the major ubiquinone. This yeast was found to represent a new species of *Candida* and is described in the present paper.

MATERIALS AND METHODS

Strain employed. The strain NK-53 employed in the present study was isolated from insect frass of a Japanese maple by direct streaking on YM agar plates containing 100 μg/m*l* of chloramphenicol and 2 mg/m*l* of sodium propionate. The pH of the medium was adjusted to 4.5.

Investigation of taxonomic criteria traditionally employed. Most of the methods employed for the examination of morphological, physiological, and biochemical characteristics were those described by van der Walt and Yarrow (9). Assimilation of nitrogen compounds was investigated on solid media with starved inoculum. Vitamin requirements were investigated according to Komagata and Nakase (4). The maximum growth temperature was determined in YM broth using metal block baths.

Investigation of chemotaxonomic criteria. Extraction and purification of ubiquinones were carried out according to Nakase and Suzuki (7) using cells harvested in the stationary phase. Ubiquinone isoprenologues were identified by high performance liquid chromatography (HPLC). DNAs were isolated and purified according to Nakase and Suzuki (6). DNA base composition was analyzed by HPLC after hydrolysis of DNA with nuclease and phosphatase as described by Hamamoto and Nakase (2). DNA-DNA reassociation experiment was carried out by the membrane filter technique reported by Kaneko (3) as described by Hamamoto and Nakase (2).

Monosaccharide composition of whole cell hydrolyzates was analyzed by HPLC after hydrolysis with 2 M trifluoroacetic acid as described by Suzuki and Nakase (8).

RESULTS AND DISCUSSION

The strain NK-53 was characterized by Q-8 as the major ubiquinone, glucan-mannan type cell wall, and mol% G+C of 41.6. Among species with Q-8, *C. homilentoma*, *C. silvanorum* and *Pichia burtonii* (≡*Hyphopichia burtonii*) resemble NK-53 in the physiological and biochemical characteristics.

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Table 1. DNA relatedness of *Candida* sp. NK-53 and *Candida silvanorum* JCM 1804^T

Unlabeled DNA from	Mol% G+C	% Relative binding of labeled DNA from	
		Candida sp. NK-53	*C. silvanorum* JCM 1804^T
Candida sp. NK-53	41.6	100	12
C. silvanorum JCM 1804^T	38.2	19	100
C. parapsilosis JCM 1785^T		12	3
Calf thymus		2	1

However, the mol% G+C of *C. homilentoma* and *P. burtonii* were reported to be 48.3～49.0 (1) and 35.5～36.3 (1), respectively, and clearly rejects the conspecificity of these two species with NK-53. Therefore, the DNA-DNA reassociation experiment was carried out between NK-53 and *C. silvanorum* whose mol% G+C was reported to be 41.7 (5) though a value of 38.2 was obtained by the HPLC method. As shown in Table 1, a low DNA homology value was obtained between DNAs of these two yeasts. Apparently, NK-53 represents a different species from *C. silvanorum*. A new species *C. gotoi* is proposed for NK-53.

***Candida gotoi* Nakase et Suzuki sp. nov.**

In liquido YM post dies 3 ad 25°C, cellulae vegetativae rotundae, sub-ovoideae, ovoideae aut longae, (3.5～6.5) × (3.5～7) μm, singlulae, binae, catenatae vel in pseudomycelia. Sedimentum formatur. Post unum mensem ad 17°C, cultura in agaro YM glauco-albiba, glabra, semi-nitida, mollis aut butyracea, margine glabra vel in parte ciliata. Pseudomycelium formatur. Glucosum, galactosum (lente) et sucrosum fermentantur at non maltosum, lactosum, melibiosum nec raffinosum. Glucosum, galactosum, sucrosum, maltosum, cellobiosum, trehalosum, raffinosum (tardum), melezitosum, amylum solubile, D-xylosum, L-arabinosum, D-ribosum, L-rhamnosum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum, mannitolum, glucitolum, α-methyl-D-glucosidum, salicinum, glucono-δ-lactonum, acidum 2-ketogluconicum, adicum DL-lacticum, acidum succinicum et acidum citricum assimilantur at non L-sorbosum, lactosum, melibiosum, D-arabinosum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum nec inositolum. Kalium nitricum non assimilatur. Maxima temperatura crescentiae : 36～37°C. Ad cresentiam vitaminae non necessarium est. Diazonium caeruleum B negativum. Proportio molaris guanini+cytosini in acido deoxyribonucleico : 41.6 (per HPLC). Ubiquinonum majus : Q-8

Holotypus : Isolata ex materia acquisita ex canalibus insectorum in acer Japoniae, Akabane, Chigasaki, Kanagawa Pref., Japonia, Nov. 1982, T. Nakase, JCM 10145 (originaliter NK-53) conservatur in collectionibus quas Japan Collection of Microorganisms, Wako, Saitama Pref. sustentat.

Growth in YM broth : After 3 days at 25°C, cells are round, short oval to oval or elongate, (3.5～6.5) × (3.7～7) μm and occur singly, in pairs, in chains or in pseudomycelia (Fig. 1 A). Pseudomycelial cells are 2～3 μm in diam. and up to 12 μm in length. A sediment is formed. After 1 month at 17°C, islets and a sediment are present.

Growth on YM agar : After 1 month at 17°C, the streak culture is grayish white, smooth, dull-shining, soft to butyrous. The margin is entire or in part fringed with pseudomycelia.

Dalmau plate culture on potato dextrose agar : Pseudomycelia are abundantly produced. They are well-developed (Fig. 1 B).

Sexual reproduction : Not observed.

Fermentation :

Glucose	+	Lactose	−
Galactose	+(slow)	Melibiose	−
Sucrose	+	Raffinose	−
Maltose	−		

Assimilation of carbon compounds :

Glucose	+	Ethanol	+
Galactose	+	Glycerol	+
L-Sorbose	−	Erythritol	+
Sucrose	+	Ribitol	+
Maltose	+	Galactitol	+
Cellobiose	+	D-Mannitol	+
Trehalose	+	D-Glucitol	+

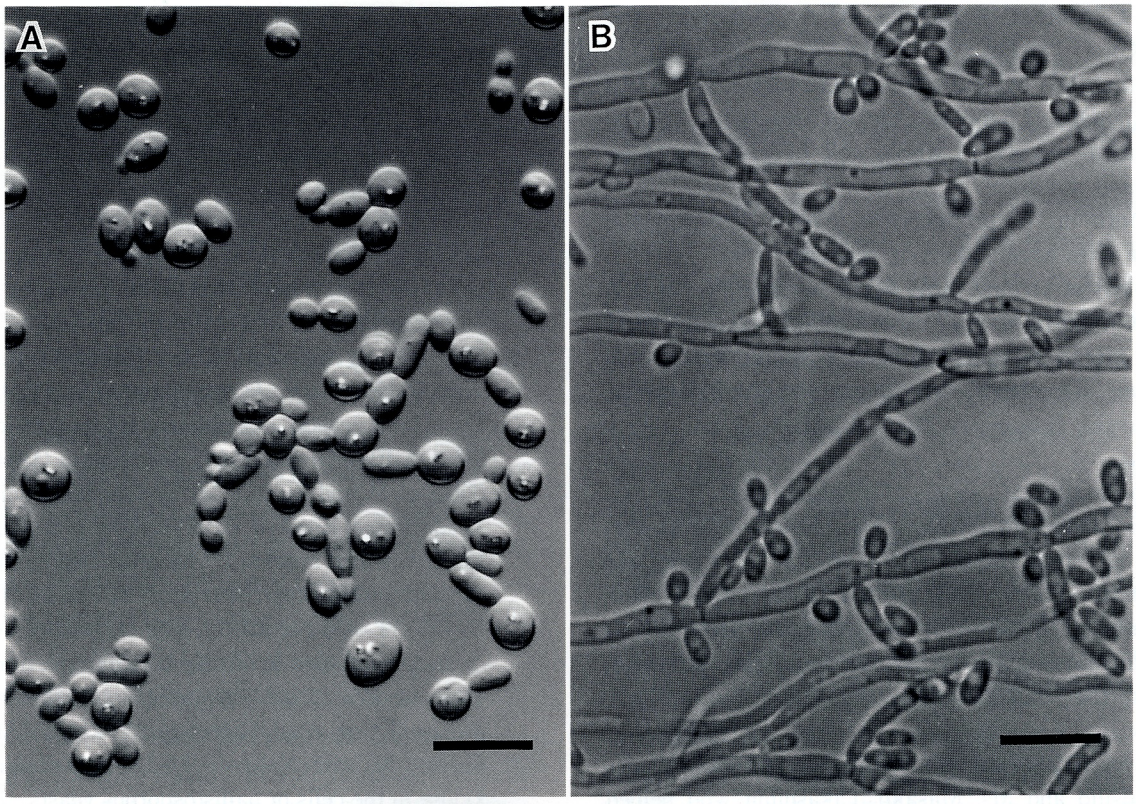

Fig. 1. Morphology of *Candida gotoi* sp. nov. NK-53

A : Vegetative cells grown in YM broth for 3 days at 25°C. Scale bar indicates 10 μm

B : Pseudomycelia developed on Dalmau plate culture with potato dextrose agar after 7 days at 25°C. Scale bar indicates 10 μm

Lactose	−	α-Methyl-D-glucoside	+
Melibiose	−	Salicin	+
Raffinose	+(slow)	Glucono-δ-lactone	+
Melezitose	+	2-Ketogluconic acid	+
Inulin	−	5-Ketogluconic acid	−
Soluble starch	+	D-Glucuronic acid	−
D-Xylose	+	D-Galacturonic acid	−
L-Arabinose	+	DL-Lactic acid	+(weak)
D-Arabinose	−	Succinic acid	+
D-Ribose	+	Citric acid	+
L-Rhamnose	+	Inositol	−

Assimilation of nitrogen compounds :

Ammonium sulfate	+	Ethylamine hydrochloride	+
Potassium nitrate	−	L-Lysine hydrochloride	+
Sodium nitrite	−	Cadaverine dihydrochloride	+

Maximum growth temperature : 36～37°C.

Vitamin required : Nil.

Production of starch-like substances : Negative.

Growth on 50% (w/w) glucose-yeast extract agar : Negative.

Growth in the presence of 100 ppm of cylohexamide : Negative.

Hydrolysis of fat : Negative.

Diazonium blue B color reaction : Negative.

Urease : Negative.

Liquefaction of gelatin : Negative.

Acid production on chalk agar : Positive (very weak).

G+C content of nuclear DNA : 41.6 mol% (by HPLC).

Ubiquinone system : Q-8 (80.7 mol%), Q-7 (17.3 mol%), Q-6 (1.5 mol%), Q-9 (0.5 mol%).

Neutral sugars in whole cell hydrolyzate : Glucose and mannose.

Riboflavin is produced in carbon assimilation media with several carbon sources.

Holotype strain : JCM 10145 (originally NK-53) is the holotype strain of this species. It was isolated from insect frass of a Japanese maple (*Acer palmatum* Thunberg), in Nov., 1982, Akabane, Chigasaki, Kanagawa Pref., Japan.

Practically, *C. gotoi* is distinguished from *C. silvanorum* by its ability to assimilate galactitol and 2-ketogluconic acid, and inability to assimilate melibiose. The specific epithet "*gotoi*" was chosen in honor of Dr. Shoji Goto, an *emeritus* professor of Yamanashi University, for his prominent contribution to the yeast taxonomy.

Acknowledgements. We thank Mr. Alfonso Mieres Hermosillo and Ms. Masako Takashima who helped with several experiments.

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タカオカエデの木喰い虫の木屑より分離した不完全酵母の新種，*Candida gotoi*

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1982年にタカオカエデ（*Acer palmatum* Thunb.）の幹の木喰い虫が穿孔した木屑から分離した1酵母，NK-53株はユビキノン系の主成分がQ-8であり，グルカン-マンナン型の細胞壁をもち，DNAのGC含量は41.6 mol%であった．この性状は *Candida silvanorum* に類似していたが，DNA相同値は19%であり両者は別種であった．NK-53株は新種と考えられたので，*Candida gotoi* と命名することを提案した．実用的には，ガラクチトールおよび2-ケトグルコン酸を資化し，メリビオースを資化できないことで，*C. gotoi* は *C. silvanorum* から識別できる．