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Physiological and DNA Characterization of *Candida maltosa*, a Hydrocarbon-Utilizing Yeast

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Abstract. Selected yeasts classified as *Candida sake* van Uden et Buckley were examined for their physiological, morphological and immunological properties and their DNA relatedness. *Candida maltosa* Komagata, Nakase et Katsuya is herein recognized as a species separate from *C. sake*. *Candida maltosa* was distinguished from *C. sake* and from *C. tropicalis* by insignificant DNA reassociation. In addition, *C. maltosa* was distinguished from *C. sake* by its higher maximal growth temperature and lower guanine plus cytosine content of its DNA and from *C. tropicalis* by its failure to utilize soluble starch for growth and its resistance to cycloheximide. The species *C. cloacae* and *C. subtropicalis* are placed in synonymy with *C. maltosa*.

Key words: *Candida maltosa* – *C. tropicalis* – *C. cloacae* – *C. subtropicalis* – *C. sake* – DNA Relatedness – GC Contents – Yeasts Hydrocarbon Utilization – Serology of Yeasts.

A variety of yeasts, particularly species of *Candida*, are known to assimilate alkanes (Komagata *et al.*, 1964; Klug and Markovetz, 1967; Bos and de Bruyn, 1973). Among those giving the greatest cell crops per unit of substrate are representatives of *C. subtropicalis*, *C. tropicalis* and isolates grouped with *C. sake* by van Uden and Buckley (Lodder, 1970). These three species have similar fermentation and assimilation patterns. Bos and de Bruyn (1973) examined 29 strains included in *C. sake*. The type strain grew weakly on alkanes, whereas strains originally described by Komagata *et al.* (1964) as *C. maltosa* and *C. cloacae* grew well. Nakase and Komagata (1971) stated that these two yeasts should be treated as separate species as they grow at higher temperatures (40–42° C) than *C. sake*. Later, Nakase *et al.* (1972) distinguished *C. subtropicalis* from *C. tropicalis* by its higher GC content, agglutination reactions, proton-magnetic-resonance spectrum of the cell-wall mannan and inability to assimilate soluble starch. The assimilation and fermentation reactions described for *C. subtropicalis* coincide with those given for *C. sake* by Lodder (1970).

To determine the correct classification of the above-mentioned species, we have examined the morphological, physiological, and immunological properties and the DNA relatedness of selected isolates.

Materials and Methods

Organisms and Morphological and Physiological Characteristics. The cultures examined are listed in Table 1. The morphological and physiological characterization of the yeasts was performed according to the methods in Lodder (1970). The assimilation tests were incubated on a shaker for 21 days. Growth on hydrocarbons was determined by the method of Markovetz and Kallio (1964) and in yeast nitrogen base (Difco) with 1.0% hydrocarbon.

Immunological Characteristics. Antisera were produced in rabbits and homogenate antigen prepared according to the procedure of Stickle *et al.* (1972). A micro-immunodiffusion technique of double-diffusion in two dimensions was used as described by Ibrahim and Hammon (1968). Adsorption-in-gel was accomplished by the method of Ibrahim and Hammon (1969). The slides and templates used were identical to those employed for immunodiffusion. The central well was first filled with the adsorbing antigen, which was allowed to diffuse for 2 hrs at 35–37° C. The residual antigen was then removed by aspiration and the central well was refilled with the antiserum to be adsorbed. Simultaneously, the outer wells were filled with the test antigens and the slides were incubated at 35–37° C. Observations for precipitin bands were made at 12- to 18-hr intervals.

DNA Base Composition and DNA Reassociation. DNA was isolated and purified according to the methods previously described (Meyer and Phaff, 1969). DNA base composition (expressed as the mean molar percent of the guanine and cytosine content, %GC) was determined by the thermal denaturation method and formula [%GC = (TM – 69.3)/

Table 1. List of Cultures[a]

Organisms	Designation	Source
C. tropicalis	ATCC 750, CBS 94	Isolated from human. Type strain
C. tropicalis	ATCC 9968, CBS 2317	Russian food yeast, *Monilia murmannica*
C. tropicalis	ATCC 18807, CBS 5701	Type strain of *C. benhamii*, isolated from case of *Onychomycosis manus*
C. tropicalis	ATCC 28142, CBS 6320	Industrial strain for production of citric acid
C. tropicalis	ATCC 28724, CBS 6719	Isolated from water, Chesapeake Bay
C. tropicalis	CBS 6400	Isolated from soil in Japan
C. tropicalis	CBS 6628	Industrial strain
C. tropicalis	CBS 6632	Industrial strain
C. tropicalis	GSU 231	Isolated from human
C. tropicalis	GSU 1081	Isolated from human
C. tropicalis	GSU 5338	Isolated from oil, received from Nakase as AJ 5338
C. sake	ATCC 14478, CBS 159	Type strain, isolated from saké-moto
C. sake	ATCC 28136, CBS 617	Type strain of *C. tropicalis* v. *lambica*, isolated from lambic beer
C. sake	ATCC 28137, CBS 2219	Type strain of *C. oleophila*, isolated from olives
C. sake	ATCC 28138, CBS 2920	Authentic strain of *C. vanriji*, from soil in Sweden
C. sake	ATCC 28139, CBS 2935	Type strain of *C. natalensis*, isolated from soil in Natal
C. sake	ATCC 22021, CBS 5690	Type strain of *C. salmonicola*, isolated from frozen salmon stick
C. sake	ATCC 28141, CBS 5740	Isolated from soil in Chile
C. sake	ATCC 28140, CBS 5611	Type strain of *C. maltosa*, isolated from adhesives in mono-sodium glutamate tank
C. sake	ATCC 20184, CBS 5612	Type strain of *C. cloacae*, isolated from mud
C. sake	ATCC 28721, CBS 6716	Isolated from water, Chesapeake Bay
C. sake	ATCC 28722, CBS 6717	Isolated from sediment, Eastern Bay, Chesapeake Bay
C. sake	ATCC 28723, CBS 6718	Isolated from water, Chesapeake Bay
Candida sp.	ATCC 20275	Deposited at ATCC by Kanegafuchi Chemical Industry Co., Ltd. Osaka, Japan, as C. novellus
Candida sp.	CBS 6658	Deposited at CBS by Kanegafuchi Chemical Industry Co., Ltd. Japan, as C. novellus
Candida sp.	ATCC 20275	Received from Liquichimicia Biosintesi S.p.A. Milan, Italy, as C. novellus
C. subtropicalis	ATCC 28241, CBS 6465	Type strain, isolated from air
C. subtropicalis	ATCC 28284, CBS 6680	Isolated from soil
C. subtropicalis	GSU 42	Isolated from oil slick, Georgia, U.S.A.

[a] ATCC = American Type Culture Collection; CBS = Centraalbureau voor Schimmelcultures; GSU = Georgia State University.

0.41] of Marmur and Doty (1962). DNA from *Candida parapsilosis* type culture (ATCC 22019) was used as the standard DNA ($T_m = 85.9°$ C). The DNA reassociation techniques employed were according to Denhardt (1966) and Johnson and Ordal (1968) with modifications of Meyer and Phaff (1972). Labeled DNA was extracted from a culture grown for 14 to 24 hrs in 5% glucose in Yeast Nitrogen Base (Difco) supplemented with 2 μc/ml uracil -6-H^3 and 2 μc/ml adenine -8-H^3. DNA was sheared by passing the sample two times through a French mini pressure cell at 20000 psi. Vials containing one 1-cm filter with approximately 25 μg of immobilized single-stranded DNA were incubated with preincubation medium (PM) at 65° C for $2^1/_2$ hrs. The PM was removed and 1.0 μg of fragmented, labeled DNA in 0.05 or 0.1 ml of 2×SSC (SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0 ± 0.2) was added to each vial. Incubation was continued at 65° C for 12 hrs. Filters were washed in 2×SSC at 65° C, dried for several hours at 37° C and cooled before the addition of scintillation fluid (Spectrafluor PPO-POPOP Amersham/Searle) prepared in toluene–Triton X-100 (2 : 1 v/v).

Relatedness was calculated from the following equation: [(disintegrations per minute (dpm) of bound H^3-DNA in the heterologous reaction)/(dpm of bound H^3-DNA in the homologous reaction)] × 100 = percentage of genetic relatedness.

Results

The physiological characteristics of the yeasts permitted their separation into three groups (Table 2). Nine of the cultures were similar to the type strain (CBS 159) of *Candida sake*. These yeasts all had maximal growth temperatures of less than 34° C, failed to utilize soluble starch and typically gave weak to latent fermentations of galactose, maltose and trehalose. Six isolates were grouped with the type strain of *C. maltosa* CBS 5611. These cultures, which included the type cultures of *C. cloacae* and *C. subtropicalis*, fermented maltose weakly, failed to utilize soluble starch and had maximal growth temperatures of 40–42° C. The cultures of *C. tropicalis* were distinct in that they all

Table 2. Physiological properties of tested strains[a]

Candida sake										
CBS	159	617	2219	2920	2935	5690	5740	6716	6717	6718
ATCC	14478	28136	28137	28138	28139	22021	28141	28721	28722	28723
Fermentation										
Glucose	+	+	+	+	+	+	+	+	+	+
Galactose	w	w	+	l	l	l	w	l	w	+
Sucrose	+	l	w	w	w	w	+	l	l	−
Maltose	l	w	w	w	w	+	+	l	l	−
Lactose	−	−	−	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−	−	−	−
Trehalose	l	l	−	l	−	l	l	−	w	w
Assimilation										
Glucose	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
L-Sorbose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	−	+	+	+	l	+	+	l	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	−	−	−	−	−	−	−
Melibiose	−	−	−	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−	−	−	−
Melezitose	+	+	+	+	+	+	−	+	+	+
Inulin	−	−	−	−	−	−	−	−	−	−
Sol. starch	−	−	−	−	−	−	−	−	−	−
D-Xylose	+	+	+	+	l	+	+	+	+	+
L-Arabinose	−	−	−	−	−	−	−	−	−	−
D-Arabinose	−	−	−	−	−	−	−	−	−	−
D-Ribose	−	−	l	−	l	−	−	−	−	−
L-Rhamnose	−	−	−	−	−	−	−	−	−	−
Glycerol	l	+	+	+	+	+	+	+	l	+
Erythritol	−	−	−	−	−	−	−	−	−	−
Ribitol	−	l	l	+	l	l	+	+	l	l
Galactitol	−	−	−	−	−	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+	+	+	+	+
D-Glucitol	+	+	+	+	+	+	+	+	+	+
α-Methylglucoside	+	l	l	+	−	+	+	+	+	l
Salicin	l	l	+	+	l	+	+	+	l	+
Arbutin	l	l	+	l	+	−	l	l	l	+
Lactate	−	l	l	l	−	+	l	+	l	l
Succinate	+	+	l	+	+	+	+	+	+	+
Citrate	−	l	+	l	l	+	+	+	−	+
Glucono-δ-lactone	+	l	+	+	+	+	−	l	+	+
Keto-2-gluconate	+	+	+	+	+	+	+	+	+	+
Keto-5-gluconate	−	−	l	−	+	−	−	−	−	l
Inositol	−	−	−	−	−	−	−	−	−	−
Max. temp. for growth, °C	28−30	28−30	28−30	28−30	28−30	28−30	28−30	30−32	30−32	32−34
Growth without vitamins	−	+	−	+	−	−	−	+	+	+
Growth on n-Hexadecane	+	+	+	+	+	+	+	+	+	+
Growth on n-Octane	+	+	+	+	+	+	+	+	+	+
Fat splitting	−	−	−	−	−	−	+	nd	nd	nd

[a] Symbols for reactions: + = positive, − = negative, l = latent, w = weak, nd = not determined.

utilized soluble starch as a sole source of carbon for growth. In other studies in our laboratories (GSU and CBS) of over 240 isolates, we have found starch (only Difco) utilization to be a reliable character in the routine identification of *C. tropicalis*. In addition, it was noted (GSU) that cultures classifiable as *C. maltosa* (15 isolates all from oil bearing soils or waters) were resistant to 0.4 g per liter of cycloheximide at 25° C. Cycloheximide resistance is rarely found among wild-type isolates of *C. tropicalis*.

Table 2 (continued)

Candida maltosa						
CBS ATCC	5611 28140	5612 20184	6465 28241	6658 20275	6680 28284	GSU 42
Fermentation						
Glucose	+	+	+	+	+	+
Galactose	−	l	+	+	+	w
Sucrose	+	+	+	+	+	+
Maltose	l	l	l	w	w	w
Lactose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Trehalose	+	+	+	+	+	+
Assimilation						
Glucose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
L-Sorbose	+	l	+	+	l	l
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Cellobiose	l	+	l	l	l	+
Trehalose	+	+	+	+	+	+
Lactose	−	−	−	−	−	−
Melibiose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Melezitose	+	+	+	+	+	+
Inulin	−	−	−	−	−	−
Sol. starch	−	−	−	−	−	−
D-Xylose	+	+	+	+	+	+
L-Arabinose	−	−	−	−	−	−
D-Arabinose	−	−	−	−	−	−
D-Ribose	−	−	−	−	−	−
L-Rhamnose	−	−	−	−	−	−
Glycerol	+	+	+	+	+	+
Erythritol	−	−	−	−	−	−
Ribitol	+	l	+	+	l	l
Galactitol	−	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+
D-Glucitol	+	+	+	+	+	+
α-Methylglucoside	+	+	+	+	+	+
Salicin	l	+	l	l	l	l
Arbutin	+	+	+	+	+	+
Lactate	l	−	l	l	−	−
Succinate	+	+	+	+	+	+
Citrate	+	+	−	+	l	nd
Glucono-δ-lactone	+	+	+	+	+	nd
Keto-2-gluconate	+	+	+	+	+	nd
Keto-5-gluconate	+	+	+	+	l	nd
Inositol	−	−	−	−	−	−
Max. temp. for growth, °C	40−41	38−39	40−41	40−41	40−42	41−42
Growth without vitamins	−	−	−	−	−	−
Growth on n-Hexadecane	+	+	+	+	+	+
Growth on n-Octane	+	+	+	+	+	+
Fat splitting	+	+	+	+	+	+

Selected cultures from the *C. tropicalis* and *C. maltosa* groups were used in the immunological studies. The cultures were compared with a culture of *C. albicans* serotype A as this type is reported to be serologically similar to *C. tropicalis* by agglutinin reactions (Sweet and Kaufman, 1970). The antigenic groupings based on precipitin reactions are presented in Table 3. These groupings are distinct from agglutinin groupings developed by other researchers. *C. maltosa* (*C. subtropicalis* CBS 6465) lacked antigens present in *C. tropicalis* and *C. tropicalis* contained two serotypes. These findings are in agreement with Nakase

Table 2 (continued)

Candida tropicalis										
CBS ATCC	94 750	5701 18807	6320 28142	6400	6628	6632	6719 28724	2317 9968	(GSU 231)	(GSU 1081)
Fermentation										
Glucose	+	+	+	+	+	+	+	+	+	+
Galctose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−	−	−	−
Trehalose	+	+	+	+	+	+	+	l	+	+
Assimilation										
Glucose	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
L-Sorbose	l	l	l	−	l	+	l	l	l	l
Sucrose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	l	+	l	+	l	l	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	−	−	−	−	−	−	−
Melibiose	−	−	−	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−	−	−	−
Melezitose	+	+	+	+	+	+	+	+	+	+
Inulin	−	−	−	−	−	−	−	−	−	−
Sol. starch	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+
L-Arabinose	−	−	−	−	−	−	−	−	−	−
D-Arabinose	−	−	−	−	−	−	−	−	−	−
D-Ribose	−	l	−	l	−	−	−	−	−	−
L-Rhamnose	−	−	−	−	−	−	−	−	−	−
Glycerol	l	+	l	+	l	−	−	+	+	+
Erythritol	−	−	−	−	−	−	−	−	−	−
Ribitol	+	+	+	+	+	l	+	+	+	+
Galactitol	−	−	−	−	−	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+	+	+	+	+
D-Glucitol	+	+	+	+	+	+	+	+	+	+
α-Methylglucoside	+	+	+	+	+	+	+	+	+	+
Salicin	−	+	l	−	l	l	l	l	l	−
Arbutin	l	+	l	l	l	l	l	l	l	nd
Lactate	+	+	+	−	+	+	+	l	nd	nd
Succinate	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	l	nd	nd
Glucono-δ-lactone	+	+	+	+	+	+	+	+	nd	nd
Keto-2-gluconate	+	+	+	+	+	+	+	+	nd	nd
Keto-5-gluconate	+	+	+	+	+	+	+	+	nd	nd
Inositol	−	−	−	−	−	−	−	−	−	−
Max. temp. for growth, °C	42−43	41−43	43−45	40−42	41−43	41−43	42−43	41−43	41−43	40−42
Growth without vitamins	−	−	−	−	−	−	−	−	−	−
Growth on n-Hexadecane	+	+	+	+	+	+	+	+	+	+
Growth on n-Octane	+	+	+	+	+	+	+	+	w	w
Fat splitting	+	+	+	+	+	+	+	+	+	+

et al. (1972). Antisera to CBS 6465 gave lines of identity with the homologous antigen and antigens of CBS 5611 and CBS 5612 further indicating the similarity of *C. maltosa*, *C. cloacae* and *C. subtropicalis*.

The DNA base composition was determined for 19 of the physiologically grouped (Table 2) *Candida* strains (Table 4). The %GC spanned the narrow range of 35.1−41%. The three strains of *C. tropicalis* had GC values at the lower end of the range (35.1−36.1%) and the 10 isolates of *C. sake* demonstrated %GC at the upper end of the range (37.8−41%). The 6 isolates grouped as *C. maltosa* had GC values (35.6−36.8%)

Table 3. Antigenic relationships of selected isolates of *Candida* using precipitin reactions

Species	Antigens recognized[a]
C. tropicalis GSU 5338	1 2
C. tropicalis GSU 1081	1 2 3
C. maltosa CBS 6465	1
C. albicans serotype A	1 2 5 6

[a] Numbers denote patterns of precipitin reactions for maltose-fermenting species of *Candida* as related to *C. albicans* serotype A.

Table 4. DNA base composition of selected strains of *Candida*[a]

Organism and designation	T_m + standard deviation	%GC
C. tropicalis ATCC 750[b]	84.1 ± 0.20	36.1
C. tropicalis ATCC 28142	83.7 ± 0.15	35.1
C. tropicalis ATCC 18807	84.0 ± 0.07	35.9
C. maltosa ATCC 28140[b]	84.0 ± 0.15	35.9
C. maltosa ATCC 28241	83.9 ± 0.05	35.6
C. maltosa GSU 42	84.2 ± 0.15	36.3
C. maltosa ATCC 20184	84.3 ± 0.31	36.6
C. maltosa ATCC 20275	84.3 ± 0.10	36.6
C. maltosa ATCC 28284	84.4 ± 0.08	36.8
C. sake ATCC 14478[b]	85.7 ± 0.29	40.0
C. sake ATCC 28723	84.8 ± 0.08	37.8
C. sake ATCC 28139	84.8 ± 0.18	37.8
C. sake ATCC 28722	84.9 ± 0.07	38.0
C. sake ATCC 28721	85.2 ± 0.21	38.8
C. sake ATCC 28136	85.2 ± 0.12	38.8
C. sake ATCC 28138	85.5 ± 0.10	39.5
C. sake ATCC 22021	85.6 ± 0.05	39.8
C. sake ATCC 28137	86.0 ± 0.10	40.7
C. sake ATCC 28141	86.1 ± 0.16	41.0

[a] Expressed as %GC (mean molar percent of guanine plus cytosine). At least three T_m determinations were done on each DNA sample to obtain the average T_m value and standard deviation.
[b] Type culture of species.

similar to and higher than *C. tropicalis* but lower than *C. sake*. To determine further the extent of relatedness between these yeasts, DNA reassociation studies were undertaken (Table 5). A high degree of DNA reassociation (86–100%) was demonstrated between the DNA from strains physiologically grouped (Table 2) as *C. maltosa* and reference DNA from *C. maltosa* ATCC 28241 (type culture of *Candida subtropicalis*) and reference DNA from *C. maltosa* (ATCC 20275 received as Candida novellus). No significant DNA reassociation was revealed between the DNA from strains of *C. maltosa* and reference DNAs from *C. sake* (type culture) or *C. tropicalis* (type culture).

Table 5. DNA relatedness of *Candida maltosa* ATCC 28241 and ATCC 20275, *Candida sake* ATCC 14478 and *Candida tropicalis* ATCC 750 to various strains of *Candida*

Source of unlabeled DNA	% relative binding of DNA from			
	C. maltosa		*C. sake*	*C. tropicalis*
	ATCC 28241[a]	ATCC 20275[a]	ATCC 14478[a]	ATCC 750[a]
C. maltosa ATCC 28241	100	100	12	27
C. maltosa ATCC 28284	100			30
C. maltosa ATCC 20184	99	97	11	11
C. maltosa ATCC 20275	91	100	11	13
C. maltosa ATCC 28140	86	100	17	32
C. maltosa GSU 42	100			
C. sake ATCC 14478	11	20	100	12
C. sake ATCC 28141		8		21
C. sake ATCC 28722		20	100	18
C. sake ATCC 28138		7	99	18
C. sake ATCC 28136		7	100	17
C. sake ATCC 28723				17
C. sake ATCC 28137		14		14
C. sake ATCC 28139		9	100	14
C. sake ATCC 28721		13	100	12
C. tropicalis ATCC 750	26	21	14	100
C. tropicalis ATCC 18807				100
C. tropicalis ATCC 28142		25		88
Escherichia coli (non-specific control)	7	3	6	5

[a] Source of labeled DNA.

Discussion

Traditional criteria based on differences in morphology and in patterns of fermentation and assimilation of carbohydrates have been employed successfully for the distinction of numerous yeast species. Still, it is recognized that such properties reflect only a small portion of a genome. Therefore, there always may be questions as to the taxonomic validity of species distinguished by only few characteristics. The evaluation of relatedness based on the total genetic material is now recognized as a very valuable method for the comparison of microorganisms. Herein, we have correlated differences in maximal temperature for growth, ability to utilize soluble starch as a sole source of carbon, rate and degree of fermentation, and cycloheximide resistance with antigenic differences and with the distinctive nature of DNA.

All the evidence, in particular DNA reassociations, demonstrates that *Candida cloacae* Komagata, Nakase et Katsuya, *C. maltosa* Komagata, Nakase et Katsuya and *C. subtropicalis* Nakase, Fukazawa et Tsuchiya are synonymous and distinct from *C. sake* (Saito et Ota) van Uden et Buckley and *C. tropicalis* (Cast.) Berkhout. *C. cloacae* and *C. maltosa* were described in the same publication and have priority over the

later binomial *C. subtropicalis*. We have chosen *C. maltosa* as the name of this species in preference to *C. cloacae*, the type strain of which does not agree with its original description.

C. maltosa and *C. tropicalis* appear to occupy separate environmental niches. *C. tropicalis* is associated with warm-blooded animals and is frequently found in sewage and polluted waters. *Candida maltosa* has been reported from air, soil, and water, particularly if enriched with hydrocarbons, but not from humans. Berner *et al.* (1975) investigated the survival of *C. maltosa* (*C. subtropicalis*) in fresh water and estuarine habitats. They found this species maintained itself in oil-enriched sites, but did not spread to adjacent hydrocarbon-free areas.

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