Leucosporidium gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*[1]

J. W. FELL, ADELE C. STATZELL,
INGRID L. HUNTER AND H. J. PHAFF

Institute of Marine Sciences, Univ. of Miami, Fla., and Dept. of Food Science and Technology, Univ. of Calif., Davis, Calif., U.S.A.

FELL, J. W., STATZELL, A. C., HUNTER, I. L. and PHAFF, H. J. 1969. *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. Antonie van Leeuwenhoek **35**: 433–462.

Investigation of yeasts from Antarctic regions revealed that certain species of *Candida* have heterobasidiomycetous life cycles. Two distinct but overlapping groups of species were found: heterothallic and self-sporulating species. *Candida scottii* is a heterothallic species with the following life cycle: opposite mating types will conjugate and develop a dikaryotic mycelium with clamp connections. Karyogamy occurs in the teliospore which germinates and produces a promycelium. Meiosis takes place in the promycelium, followed by development of haploid sporidia to complete the life cycle. In addition, *C. scottii* has a self-sporulating phase. From a single cell, in the apparent absence of mating, a uninucleate mycelium is produced that lacks clamp connections. Teliospores, promycelia and sporidia develop that appear similar to those produced from dikaryotic mycelium.

The self-sporulating species have life histories similar to the self-sporulating phase of *C. scottii;* except that heterothallism has not been observed.

Based on these life histories the new genus *Leucosporidium* is proposed with two heterothallic species (*Leu. scottii* and *Leu. capsuligenum*) and five self-sporulating species (*Leu. antarcticum, Leu. frigidum, Leu. gelidum, Leu. nivalis* and *Leu. stokesii*). *Leu. antarcticum* and *Leu. stokesii* have not been described under the genus *Candida.*

INTRODUCTION

The imperfect yeasts generally are considered to be asexual ascomycetes, either forms whose sexual cycle has not been observed or organisms that have lost their sexual capacities. There have been, however, indications that certain yeasts may be basidiomycetes. The indicators have included the presence of ballistospores (Lodder and Kreger-van Rij, 1952), the base composition of DNA (Nakase and Komagata, 1968; Stenderup and Bak, 1968; Storck, Alexopoulos and Phaff, 1969), and the components of extracellular polysaccharides (Slodki, Wickerham and Bandoni, 1966). The first direct evidence

[1]) Contribution No. 1138 from the Institute of Marine Sciences, University of Miami, Fla.

of a basidiomycetous life cycle in yeasts was that of Banno (1967) who found that certain strains of *Rhodotorula glutinis*, when mated, followed a sexual cycle similar to that of some species belonging to the heterobasidiomycetes. Banno described the genus *Rhodosporidium* with the single species *Rhodosp. toruloides*. Subsequently, Newell and Fell (1970) described a second species, *Rhodosp. sphaerocarpum*, with a similar life history.

During our investigation of yeasts from the Antarctic seas we found that certain *Candida* species have life histories similar to that of *Rhodosporidium*. In the following report we are describing a new genus *Leucosporidium* with seven species and are presenting the preliminary results of a study of their life cycles.

METHODS AND MATERIALS

Cultures studied. Collections were made during the Jan-Feb 1966 cruise of the U.S. Coast Guard Cutter *Eastwind* (Fig. 1) and during four cruises of the

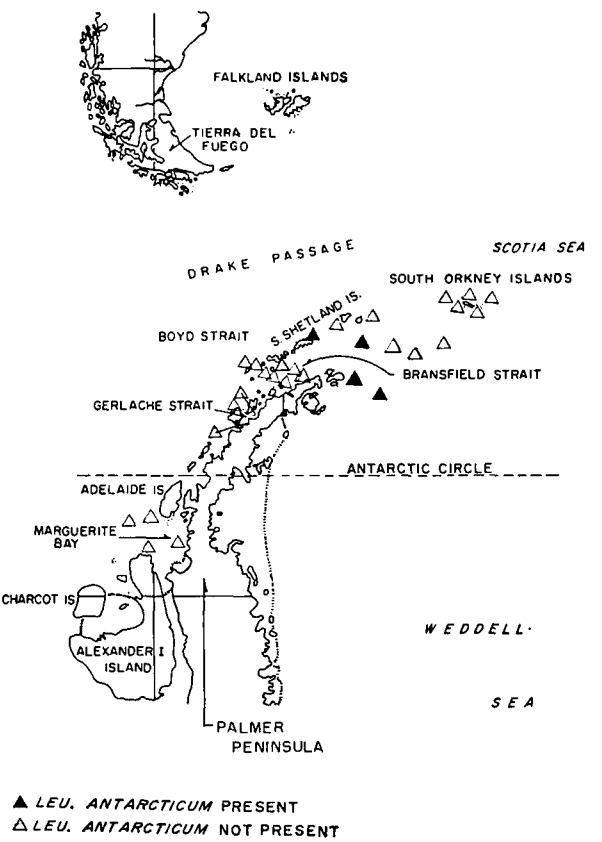


Fig. 1. Station locations of the U.S. Coast Guard Cutter *Eastwind* cruise.

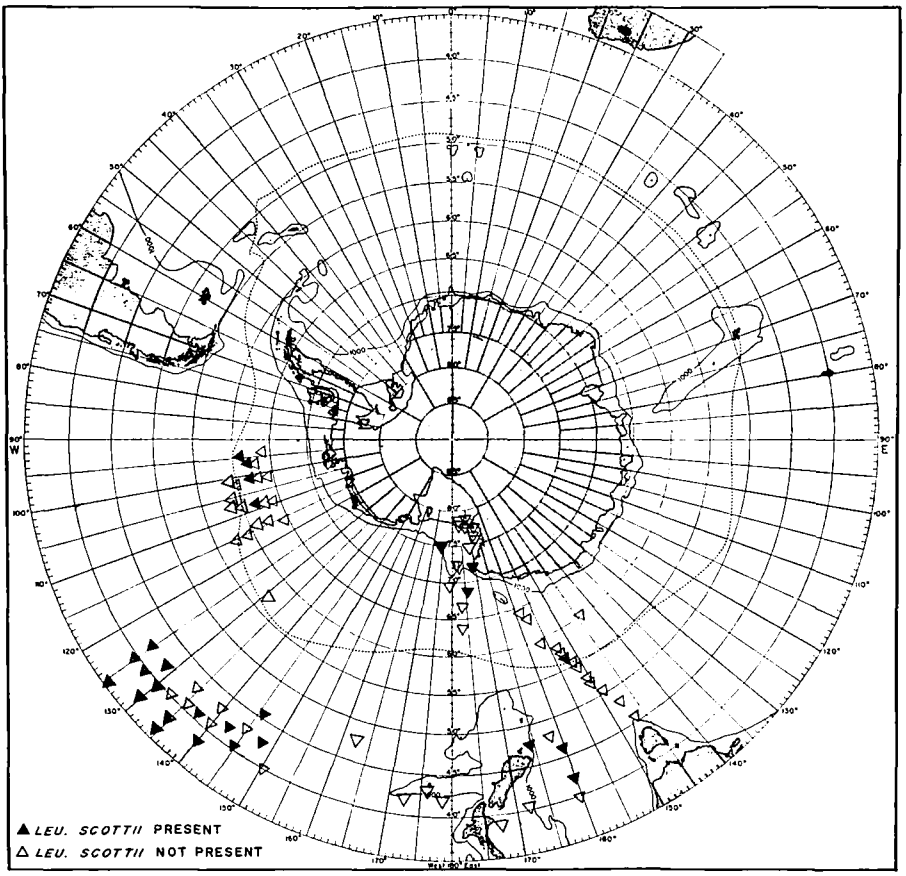


Fig. 2. Station locations of the U.S.N.S. *Eltanin* cruises.

U.S. Naval Ship *Eltanin:* Cruise 23, April–May 1966; Cruise 24, July–Aug 1966; Cruise 26, Nov–Dec 1966; and Cruise 27, Jan–Feb 1967 (Fig. 2). Two-liter samples were taken at selected depths from surface to bottom waters with an aseptic sampler (Niskin, 1962). Each sample was divided into 500-ml and 1000-ml portions and filtered through cellulose acetate membranes with a porosity of 0.45μ. The membranes were placed on agar plates (2.3% Difco nutrient agar, 2% glucose and 0.1% yeast extract in filtered sea water with the pH adjusted to 4.5 with HCl) and the plates were incubated at 12 C. After 1–3 weeks the colonies were enumerated, and selected types sub-cultured to agar slants. The cultures were maintained at approximately 5 C and returned to the Institute of Marine Sciences for study.

Other cultures were obtained from Dr. Margaret di Menna, Dept. of Agri-

culture, Hamilton, New Zealand; Dr. L. J. Wickerham, Northern Regional Research Laboratory (NRRL), U.S. Dept. of Agriculture; Mr. David Yarrow, Centraalbureau voor Schimmelcultures (CBS), Delft, The Netherlands; and Dr. J. L. Stokes, Washington State University, Pullman, Wash.

Leu. antarcticum. Forty-six isolates were examined. All were obtained from marine waters in the vicinity of the Antarctic Peninsula during the cruise of the USCGC *Eastwind.* Six of the cultures were collected 14 Feb 66, from a station (63°45'S, 53°10'W) in the Weddell Sea where the water depth was 539 m and the temperature in the water column ranged from –0.19 tot –1.70 C, salinity from 33.79 to 34.62‰. Thirty-six cultures were collected 15 Feb 66 off the tip of Joinville Island (63°30'S, 55°00'W), also in the Weddell sea. The depth of the bottom was 130 m, and the temperature range –1.44 to –1.54C, salinity 34.23 to 34.39‰. Three cultures were obtained 16 Feb 66 north of the Weddell Sea and east of the Bransfield Straits (62°08'S, 54°21'W). Depth of bottom 402 m, temperatures of the surface to bottom waters ranged –0.90 to –1.68 C, salinity 34.15 to 34.65‰. One isolate was collected off the tip of King George Island (62°08'S, 57°53'W) on 19 Feb 66. The bottom depth was 152 m, temperature range 0.67 to 0.99 C, salinity 33.84 to 34.23‰.

Leu. capsuligenum. Three strains were studied. One strain (CBS–4736) represents the culture originally described by van der Walt and van Kerken (1961) as *Torulopsis capsuligenus.* It came from the laboratory of a South African winery; further details were not available. A second strain (CBS–4381) came from Beech in Bristol (England) who had isolated it from cider. The third strain (CBS–1906) was sent to the CBS culture collection in 1953 as *T. alba.* It came from the Nagao Institute in Tokyo under No. N.I. 7350 and had been isolated from sake-moto of Kiku-Masamune.

Leu. frigidum. Two isolates were examined. P–8 (CBS–5916) was isolated from Antarctic snow by Sinclair and Stokes (1965). 5AI (CBS–5270) was isolated from Antarctic snow at Scott Base, Antarctica (di Menna, 1966*b*).

Leu. gelidum. Strain 2AH10 (CBS–5272) was isolated by di Menna (1966*b*) from soil collected at Scott Base, Antarctica.

Leu. nivalis. Strain 2AH2 (CBS–5266) was isolated by di Menna (1966*b*) from soil collected at Scott Base, Antarctica.

Leu. scottii. 65 cultures were examined. 59 strains were obtained in the Pacific sector of the Antarctic seas in the region from approximately 34° to 75° S and 100° to 150° W in water depths of 3 to 3961 meters. The water temperatures ranged from –0.34 to 14.96 C and salinities from 33.96 to 34.94‰. Three strains were isolated by di Menna (1966*b*): 4EG5 from soil; 2–51M1 from sea water; 3–AH16 from Antarctic soil. Two cultures Y–1427 and Y–5712 were obtained from Wickerham. CBS–614 is the type culture of *Candida scottii* and was

collected by Scott from soil near a meatworks at Townsville, Queensland, Australia (Lodder and Kreger-van Rij, 1952).

Leu. stokesii. Strain P–16 (CBS–5917) was isolated from an Antarctic snow core sample by Sinclair and Stokes (1965).

Species identifications and life histories. Characterizations followed the techniques recommended by Wickerham (1951), Lodder and Kreger-van Rij (1952), and Ahearn et al. (1960).

The methods for studying the life cycles varied with the species, and the specific techniques are included in the description of each species. In general the cultures were grown at 12 C on either malt agar (5% Fleischmann's diastatic dry diamalt, 2% agar in distilled water) or corn meal agar (1.7% Difco corn meal agar) plates. With the latter the Dalmau (Lodder and Kreger-van Rij, 1952) technique was employed. Teliospores formed after 1 to 3 weeks. Pieces of agar with teliospores were dissected from the plate and placed in sterile distilled water and stored at 12 C for 2 to 10 weeks. The teliospores were then streaked on aqueous agar plates (2% agar in distilled water) and incubated at 5 or 12 C. Germination was usually observed after one week. The most notable exception to this procedure was *Leu. scottii*, whose entire life cycle was observed on corn meal agar at room temperature without any transferring steps.

The nuclear staining procedure followed the Pontefract and Miller (1962) modification of the Robinow (1961) technique.

RESULTS

Two distinct but overlapping groups of species were found: heterothallic and self-sporulating species. The heterothallic species are *Leu. scottii* and *Leu. capsuligenum*. The life cycle of *Leu. scottii* (Fig. 3) is essentially as follows: mating types α and *a*, when mixed, conjugate and develop a dikaryotic mycelium with clamp connections at the septa. Karyogamy occurs in large, thick-walled teliospores which germinate and produce four-celled promycelia. In the latter structure meiosis takes place and lateral and terminal haploid sporidia develop to complete the life cycle. *Leu. scottii* in addition has a self-sporulating uninucleate phase. From a single yeast cell, in the apparent absence of mating, a uninucleate mycelium is produced that lacks clamp connections. Teliospores develop that appear similar to those developing on a dikaryotic mycelium. These teliospores germinate and produce a four-celled promycelium with lateral and terminal sporidia.

Less is known about the life cycle of *Leu. capsuligenum*. Opposite mating types conjugate to produce a dikaryotic mycelium with clamp connections and poorly developed teliospores. Germination of the spores has not been observed.

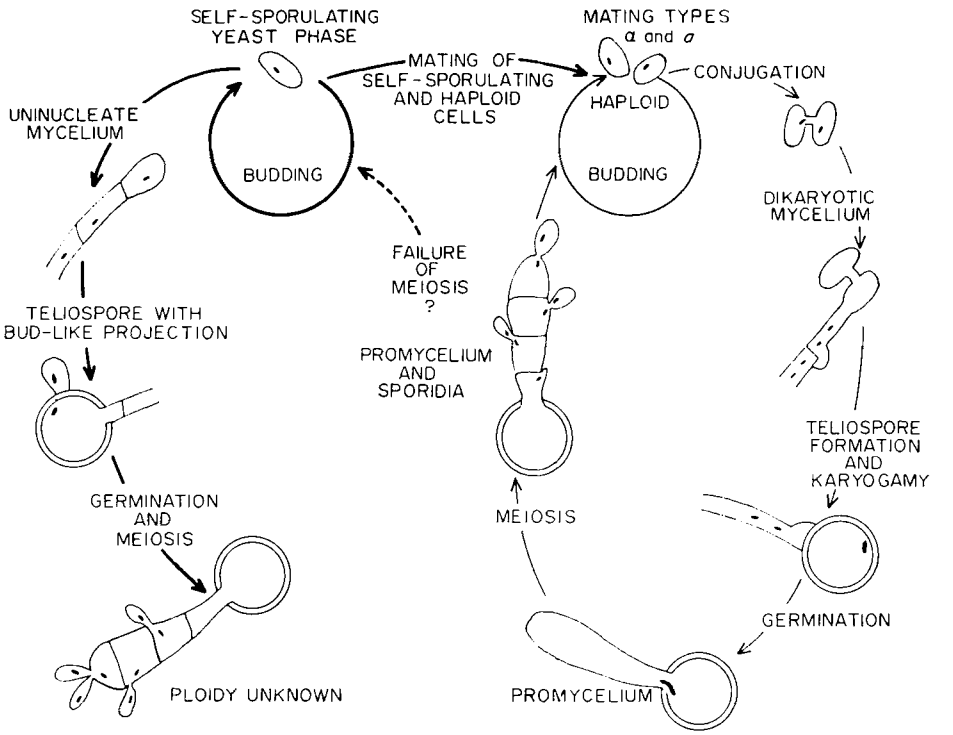


Fig. 3. Schematic representation of the life cycle of *Leucosporidium scottii*.

The exclusively self-sporulating species are *Leu. antarcticum*, *Leu. gelidum*, *Leu. frigidum*, *Leu. nivalis* and *Leu. stokesii*. Their life histories appear to be identical to the self-sporulating phase of *Leu. scottii*.

Leucosporidium gen. n. (Ustilaginales)

Status sexualis distinctus, sine soro. Aliquae species sine cellulis in statu sexuali distincto.

In primo modo reproductionis, cellulae ex α et *a* compositae; hyphae secundum copulationem cellularum haploidearum (α et *a*) formantur, binucleatae, cum septis nodosis. Teliosporae ex hyphis terminaliter aut intercalariter oriundae, saepe basi cum nodo, singulae, binae aut gregariae.

In secundo modo reproductionis hyphae producuntur e cellulis solitariis sine copulatione; hyphae uninucleatae, sine septis nodosis.

Promycelia e teliospora oriunda, cylindrica (longa vel brevia), transversim aut diagonaliter septata; sporidiae e cellulis promycelii lateraliter formantur.

Cellulae candidoideae singulae, binae, aut catenatae in propagatione vegetativa gemmas ab omni latere formant. Pseudomycelium cum blastosporis praesens interdum. In agaro malti cellulae non pigmentosae. Typus generis: *L. scottii* Fell et al.

The organisms may occur in one or several phases.

The *haploid yeast phase* consists of oval to elongate budding yeast cells and usually abundant pseudomycelial growth. True mycelium may form in some species. Growth on solid media is white to cream-colored, often mucous.

The *dikaryotic phase* develops after conjugation between opposite mating types of the haploid yeast phase. A dikaryotic mycelium is produced from one of the conjugants of each mating pair or from the conjugation tube. A clamp connection is normally present at each cross wall. The mycelium develops intercalary or terminal teliospores which are heavy-walled and granulated. Karyogamy takes place in the teliospore which germinates and produces a one- to four-celled promycelium. Reduction division takes place in the promycelium and haploid sporidia are formed laterally or terminally.

A *self-sporulating yeast phase* is also known. It consists of oval to elongate budding cells and pseudomycelium may or may not be present. The growth on solid media is white to cream-colored and often mucous. A uninucleate mycelial phase develops under suitable conditions from a single yeast cell apparently without mating. Clamp connections are not formed. Large terminal and intercalary spores, resembling the dikaryotic teliospore, are formed. The spore germinates and produces a one- to four-celled promycelium with lateral and terminal sporidia.

Ballistospores have not been found in any of the phases. Sugars may or may not be fermented, depending on the species.

Leucosporidium scottii is designated as the type species of the genus.

Key to the species of the genus *Leucosporidium*

1.	Nitrate assimilated	2
	Nitrate not assimilated	*Leu. capsuligenum*
2.	Melibiose assimilated	3
	Melibiose not assimilated	4
3.	Maltose assimilated	*Leu. gelidum*
	Maltose not assimilated	*Leu. nivalis*
4.	Rhamnose assimilated	5
	Rhamnose not assimilated	6
5.	Glucose fermented	*Leu. stokesii*
	Glucose not fermented	*Leu. scottii*
6.	Cellobiose assimilated	*Leu. frigidum*
	Cellobiose not assimilated	*Leu. antarcticum*

HETEROTHALLIC SPECIES

Leucosporidium scottii sp. n.

Imperfect stage: *Candida scottii* Diddens et Lodder 1942. Synonym of the imperfect stage: *Azymocandida scottii* (Diddens et Lodder) Novak et Zsolt 1961.

Candida scottii was originally described by Diddens and Lodder (1942) based on isolates collected by Scott (1936) in Australia from chilled beef and from soil near a meatworks. Subsequently strains were isolated by di Menna (1960, 1966*b*) from Antarctic and New Zealand soils and by Clark, Wallace and David (1954) from apples. A detailed description of *C. scottii* was given by Lodder and Kreger-van Rij (1952).

We found *C. scottii* to be abundant in the Antarctic seas but it was not present in waters adjacent to the Antarctic Peninsula (Figs. 1 and 2). By mating the various *C. scottii* isolates, including the type culture, we found the basidiomycetous life cycle depicted in Fig. 3 and discussed below. Because the life history of *Leu. scottii* has been studied in greatest detail, we are designating this species as the generic type.

Leucosporidium scottii sp. n.

Hyphae secundum copulationem cellularum (binucleatae) aut sine copulatione cellularum (uninucleatae). Teliosporae intercalares aut terminales, sphericae (7–16 μ in diametro), granulatae.

In extracto malti cellulae sunt ellipsoideae aut longovoideae (1.3–6.7) $\times$ (4.0–16) μ, singulae, binae aut catenatae. In agaro farinae maïs, pseudomycelium, mycelium verum et teliosporae sunt.

Sedimentum et annulus formantur.

Fermentatio nulla.

Glucosum, maltosum, saccharum, cellobiosum, raffinosum, L-rhamnosum et ethanolum assimilantur, at non melibiosum, inulinum, amylum nec erythritolum.

Assimilatio kalii nitratis: praesens.

Summa temperatura aucta: 15–30 C.

Ad crescentiam vitaminae externae non necessariae sunt.

Amylum non formatur.

Typus: Cultura 24–269 ex aqua oceani pacifici isolata, in CBS No. 5930, Delft, Hollandia; status sexualis alpha.

Allotypus: 24–235 (status sexualis *a*) ex aqua oceani pacifici isolata, in CBS No. 5931, Delft, Hollandia.

Paratypus: 24–220 (teliosporae formantur sine copulatione cellularum) ex aqua oceani pacifici isolata, in CBS No. 5932, Delft, Hollandia.

Growth in malt extract at 25 C: After 3 days the cells of single mating types are ovoid to elongate, (1.3–6.7) $\times$ (4.0–16.1) μ, usually single or in pairs. Reproduction is by apical budding. Strands of pseudomycelium are present.

Sediment is moderate. After one month, there is a ring, some small islets and a heavy sediment.

Growth on malt agar at 25 C: After 3 days the cells of single mating types are ovoidal to elongate (1.6–4.0) × (3.2–12.1) μ. Some strands of true and pseudomycelium are evident. After one month the growth is cream-colored, extremely mucous, and there is dense, extensive mycelium at the periphery.

Dalmau plate cultures on corn meal agar at 25 C: Single mating types after 3–5 days begin to form pseudomycelium of the candida type; after one month the mycelium bears large clusters of blastospores. True mycelium is also present.

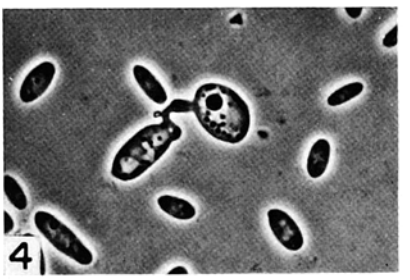
Life history: As depicted in Fig. 3 there are two distinct mycelial phases: uninucleate and dikaryotic. Both types of mycelium initiate from yeast phases: the dikaryotic from a mixture of haploid mating types α and a; the self-sporulating mycelium from a single uninucleate cell in the absence of mating. The life cycle discussed below is compiled from gross morphological observations and from nuclear stains.

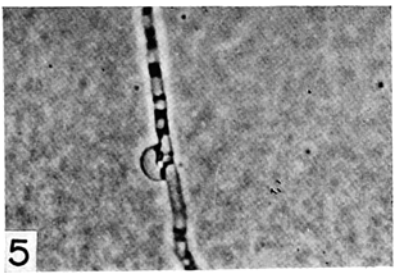
Dikaryotic phase: Heterothallic mating types α and a when mixed on corn meal agar conjugate (Fig. 4) at room temperature within 30 hr at a frequency of less than 1%. A binucleate mycelium develops from one of the conjugate cells or from the conjugation tube. After 72 hr the mycelium develops extensively with clamp connections at each septum (Fig. 5). After 4–7 days intercalary and terminal teliospores develop with one or more clamp structures between the spore and the mycelium. The teliospores (Fig. 6) are spherical, thick-walled, have a granular content and range in diameter from 7.0–16μ.

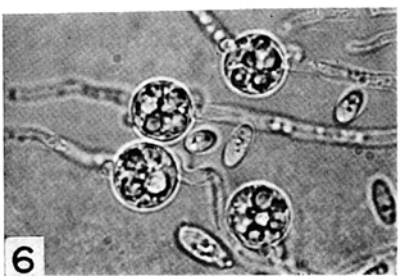
Karyogamy in the teliospore precedes germination. After 10 days the teliospore germinates and the diploid nucleus migrates into a promycelium (Fig. 7). Meiosis occurs in the promycelium which then is partitioned into four cells. Each cell contains one nucleus which divides mitotically allowing for one nucleus in the cell and one in a sporidium. Sporidia (Fig. 7) form near the septa and terminally on the distal promycelial cell. Budding of the sporidia results in mucous colonies of yeast cells representing α or a mating types.

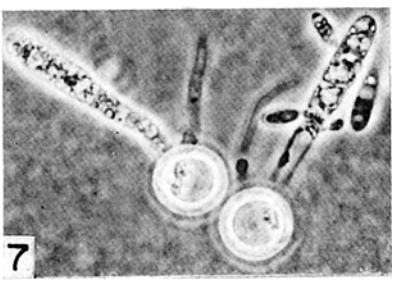
The complete life cycle was observed on either corn meal or malt agar, although the process was considerably slower on the latter. Observations of germination of teliospores and sporidial formation was facilitated by streaking two-week-old teliospores on aqueous agar.

Self-sporulating phase: The self-sporulating strains are morphologically similar to the heterothallic haploids, viz. growth on malt agar, in malt extract, and on corn meal agar. However, they exhibit a feature that is different from the haploid heterothallic strains: teliospores form on mycelium which originates from an individual uninucleate cell in the absence of mating. This mycelium does not have clamp connections, although incomplete clamp-like structures









Figs. 4, 5, 6, 7. *Leu. scottii*. (4) Conjugation between opposite mating types. (5) Mycelium with clamp connection. (6) Mycelium and teliospores in the dikaryotic phase. (7) Left: developing promycelium from a teliospore; right: four-celled promycelium with lateral sporidia. Figs. 4, 5, and 7 Phase contrast; Fig. 6 Bright field, 940 ×.

have been observed; there are blastospores or mycelial branches at the septa.

The teliospores develop from enlarged granular hyphal cells. As the cells become spheroidal the basal portion often remains intact and unswollen, forming a neck-like structure. The teliospores may have one or several bud-like projections which develop into new teliospores resulting in clusters of 2 or 3 spores. These teliospores however are not as abundant as those produced on dikaryotic mycelium. Germination and sporidial production appear to be the same as in the heterokaryotic teliospores. The ploidy of the nucleus in the various stages of the self-sporulating life history is not known.

Cells of the self-sporulating strains are able to conjugate with cells of mating types α or *a* to produce a dikaryotic mycelium with clamp connections, teliospores, promycelia and sporidia. The nuclear sequence of this type of hybrid is the same as that of a conjugant of haploid mating types; however, the ploidy of the resulting sporidia remains to be determined.

Neither the haploid nor self-sporulating phases of *Leu. scottii* mate with any other species in the genus.

Fermentation: Negative.

Assimilation of carbon compounds:

Glucose	+	D-Ribose	— or + (rarely latent)
Galactose	+ or —	L-Rhamnose	+
L-Sorbose	+	Ethanol	+
Maltose	+	Glycerol	+
Sucrose	+	Erythritol	—
Cellobiose	+	Ribitol	— or + (sometimes latent)
Trehalose	+	Galactitol	+ (rarely —)
Lactose	+ (rarely — or latent)	D-Mannitol	+
Melibose	—	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	—	DL-Lactic acid	—
Soluble starch	—	Succinic acid	—
D-Xylose	+	Citric acid	—
L-Arabinose	— or + (weak latent)	*m*-Inositol	—
D-Arabinose	— or + (sometimes latent)		

Assimilation of potassium nitrate: Positive.
Growth in vitamin-free medium: Positive.
Maximum temperature for growth: 15–30 C.
Starch formation: Negative.
Liquefaction of gelatin: Positive.
Splitting of arbutin: Positive.
Acid production on chalk agar: Negative.
Growth on 50% (w/w) glucose yeast-extract agar: Absent.
Splitting of urea: Positive.

Type culture: 24–269 (CBS–5930, mating type α) was collected in August 1966 at 129°59′W, 41°58′S at a water depth of 1364 meters, temperature of 3.0 C and salinity of 34.44‰.

Allotype: 24–235 (CBS–5931, mating type *a*) was collected in August 1966 at 134°30′W, 36°00′S, at a water depth of 1902 meters, temperature of 2.30 C, salinity of 34.62‰.

Paratype: 24–220 (CBS–5932, NRRL-Y-7104) a self-sporulating form collected in August 1966 at 134°53′W, 34°59′S, at a water depth of 155 meters, temperature of 2.59 C, salinity of 34.61‰.

Of the 59 marine Antarctic isolates, 41 are mating type *a*, 4 are mating type α, and 8 are self-sporulating forms; the sexuality of 6 isolates was not determined. Di Menna's isolates 4EG5 and 3-AH16 are type *a* and 2-51M1 is type α. Wickerham's Y-1427 is a self-sporulating form and Y-5712 is mating type *a*. CBS–614, the type culture of *Candida scottii*, is self-sporulating.

Leucosporidium capsuligenum sp. n.

Imperfect stage:[1] *Torulopsis capsuligenus* van der Walt et van Kerken 1961.

In 1961 van der Walt and van Kerken described a new species of yeast which they received via J. A. van Zyl from a South African winery. The authors pointed out that this species occupies a position intermediate between the genera *Torulopsis* and *Cryptococcus* as defined by Lodder and Kreger-van Rij (1952), because it fermented glucose, but was capsulated and produced starch. In view of the weak fermentation they placed it in *Torulopsis*.

We found by mating the three available isolates, including the type strain, that conjugation took place followed by the formation of mycelium with clamp connections. Teliospores are produced but these do not appear to develop completely. Based on the similarity in sexual behavior of this species with that of *Leu. scottii* we are including this species in *Leucosporidium*.

Leucosporidium capsuligenum sp. n.

Hyphae secundum copulationem cellularum (binucleatae). Teliosporae infrequentes non granulatae.

In extracto malti cellulae sunt subovoideae, ellipsoideae, interdum longovoideae (2.0–10.7) × (4.0–17.8) μ, singulae aut binae. Annulus et sedimentum mucosum formantur; interdum velum praesens.

In agaro farinae maïs pseudomycelium aut mycelium verum formantur secundum conjugatione cellularum haploidearum. Glucosum fermentatur (interdum tardum), maltosum non fermentatur aut tardum et exiguum; saccharum, galactosum et lactosum non fermentantur.

Glucosum, maltosum, saccharum (lente), cellobiosum (lente), ethanolum assimilantur, at non melibiosum, raffinosum, L-rhamnosum, erythritolum, nec amylum.

Kalium nitricum et natrium nitrosum non assimilantur.

Ad crescentiam vitaminae externae necessariae sunt.

Amylum formatur.

Typus: Cultura CBS No. 4736, Delft, Hollandia isolata ex cella vinorum; status sexualis alpha.

Allotypus: Cultura CBS No. 4381 (status sexualis *a*) isolata ex suco mali fermentato.

Growth in malt extract at 25 C: After 3 days the cells of single mating types are globose to ovoidal and sometimes elongate, (2.0 – 10.7) × (4.0 – 17.8) μ, occurring mainly in pairs and singly. A thin ring and a little sediment are formed. One strain (CBS–4381) produced a heavy film. After one month there

[1]) Subsequent to submitting this manuscript, Slooff (personal communication) reported that CBS-1906 was received in 1953 from Nagao Institute, Tokyo as *Torulopsis albus* Saito et Oda 1934 number N.I. 7350. In examining all available information including the characteristics of CBS-1906, Slooff concluded that CBS-1906 was a subculture of the original type strain of *T. albus* which had been used as the type strain of *Candida japonica* Diddens et Lodder 1942. Lodder and Kreger-van Rij (1952) reported that the Diddens and Lodder subculture had died. In view of this information, the imperfect stage should be listed as *C. japonica* with the synonyms *T. albus* and *T. capsuligenus*.

is a moderate to thick, slimy ring and a fairly heavy sediment. A partial or complete film may be present also.

Growth on malt agar at 25 C: After 3 days the cell sizes of single mating types are similar to those formed in malt extract except that, in addition, elongate pseudomycelial cells occur. After one month, mating types α and *a* differ in their colony morphology. Type α is mucous, glistening, and cream colored, the surface is raised, the border entire, and non-myceliated. Type *a* colonies have a soft, moist or often dry texture but are not mucous; their border is undulate and heavily myceliated. The mycelium consists of branched, septate hyphae.

Dalmau plate cultures on corn meal agar at 25 C: A typical pseudomycelium is absent. Occasionally a rudimentary pseudomycelium or a few short chains of undifferentiated, globose cells may be present.

Life history: Conjugation was observed between opposite mating types within 12 hr of mixing on corn meal agar at room temperature. After 24–48 hr, true binucleate mycelium with distinct clamp connections begins to form. After 3 weeks teliospores (Fig. 8) are rare; those observed were intercalary

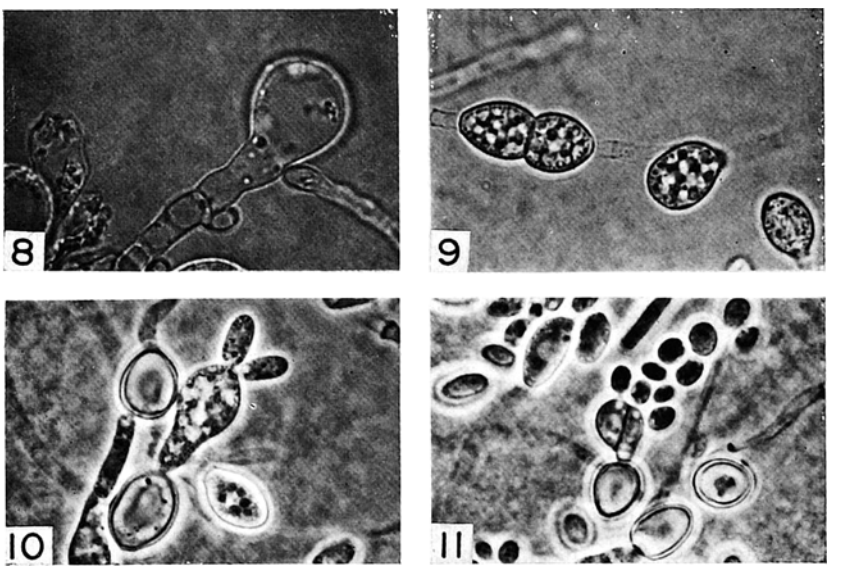


Fig. 8. *Leu. capsuligenum* teliospore with basal clamp connection. In this species the teliospores do not appear to mature. Note the lack of granular material in the spore as compared with those of other species. To the left is a shriveled teliospore. Bright field, 940 ×.

Figs. 9–11. *Leu. frigidum.* (9) Mycelium and teliospores. Bright field. (10) One-celled promycelium with terminal sporidia in strain 5AI. Phase contrast. (11) Two-celled promycelium with terminal sporidia in strain P–8. Phase contrast, 940 ×.

and terminal with clamp connections to the hyphae. These teliospores appear to be empty; in contrast, other species produce teliospores which contain large granules.

Conjugation was not observed when either mating type was mixed with isolates of the other species in the genus.

Fermentation:

Glucose	+ (sometimes latent)	Maltose	+ (weak) or —
Galactose	—	Lactose	—
Sucrose	—	Raffinose	—

Assimilation of carbon compounds:

Glucose	+	D-Ribose	+ (latent) or —
Galactose	— or + (weak or latent)	L-Rhamnose	—
L-Sorbose	—	Ethanol	+
Sucrose	+ (latent)	Glycerol	+ (sometimes latent)
Maltose	+	Erythritol	—
Cellobiose	+ (latent)	Ribitol	+
Trehalose	+	Galactitol	— or + (latent)
Lactose	—	D-Mannitol	+
Melibiose	—	D-Glucitol	+
Raffinose	—	α-Methyl-D-glucoside	+
Melezitose	—	Salicin	— or + (sometimes weak)
Inulin	—	DL-Lactic acid	—
Soluble starch	+	Succinic acid	+ (weak)
D-Xylose	+ (latent) or —	Citric acid	+ (weak)
L-Arabinose	— or + (weak latent)	*m*-Inositol	+
D-Arabinose	— or + (weak or sometimes latent)		

Assimilation of potassium nitrate: Negative.

Growth in vitamin-free medium: Absent.

(for vitamins which are required see below)

Growth at 37 C: Negative; at 30 C: Positive or negative.

Starch formation: Positive.

Gelatin liquefaction: Negative.

Splitting of arbutin: Positive or negative.

Acid formation on chalk agar: Positive (weak).

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

Splitting of urea: Positive.

Type culture: CBS–4736 (mating type α) isolated from material in a wine cellar in S. Africa (van der Walt and van Kerken, 1961).

Allotype culture: CBS–4381 (mating type *a*) isolated from cider by Beech in England.

The third strain studied (CBS–1906) is mating type *a*. It was isolated from sake-moto in Japan.

The physiological properties, based on three strains rather than the single strain available to van der Walt and van Kerken (1961), are generally in agreement with the original description of *Torulopsis capsuligenus*. The following exceptions are noted (original observations are in parentheses): the assimilation of galactose negative or weak to latent (positive); sucrose latently positive (negative); L-arabinose negative (positive); D-arabinose negative or weak and latent (positive); growth in vitamin-free medium negative (positive).

The three strains studied had different vitamin requirements. The type culture requires thiamine, but pyridoxine has a sparing action for thiamine and therefore growth is absent only in media which lack both thiamine and pyridoxine. A strain from Beech (England) requires both thiamine and pyridoxine. The third strain from the Nagao Institute (Tokyo) requires thiamine and the growth was stimulated by pantothenate.

SELF-SPORULATING SPECIES

Leucosporidium antarcticum sp. n.

This species was isolated from marine waters near the northeastern end of the Antarctic Peninsula (Fig. 1). The life history is typical of the self-sporulating forms, viz. the development of uninucleate mycelium with teliospores directly from a single yeast cell; clamp connections are not formed. Mating has not been observed in single cultures nor when isolates of *Leu. antarcticum* were intermixed or mixed with isolates of the other species of the genus *Leucosporidium*.

Leucosporidium antarcticum sp. n.

Hyphae e cellulis singulis uninucleatae, sine septis nodosis. Teliosporae intercalares aut terminales, ovoideae (5–7.5) × (5.5–10.5) μ, singulae aut brevicatenatae.

In extracto malti cellulae sunt longovoideae, (2.2–3.8) × (5.5–22) μ, singulae, binae aut brevicatenatae. Post unum mensem annulus et sedimentum formantur. In agaro farinae maïs mycelium verum cum septis sed non nodosum.

Fermentatio: nulla.

Glucosum assimilatur, at non cellobiosum, melibiosum, amylum, L-rhamnosum, erythritolum. Saccharum, maltosum, raffinosum et ethanolum non assimilantur aut exigue et lente assimilantur.

Kalium nitricum et natrium nitrosum assimilantur.

Ad crescentiam vitaminae externae non necessariae sunt.

Amylum non formatur.

Typus: Cultura AR-372 ex aqua oceani antarctici isolata, in CBS No. 5942, Delft, Hollandia.

Growth in malt extract at 12 C: After 3 days the cells are (2.2–3.8) × (5.4–21.6) μ, single, in pairs and short chains. A ring and film are not present; there is a light sediment. After one month there is a light ring, no film, and a heavy sediment.

Growth on malt agar at 12 C: After 3 days the cells are (2.2–3.8) × (5.4–24.3)μ, with a large capsule. Growth is cream colored, smooth, mucous, runny, and glistening; the border is entire and not myceliated. There is little change after one month.

Dalmau plate culture on corn meal agar at 12 C: Two types of mycelium are produced; short branched chains of pseudomycelium and long branched chains of true mycelium. After 3 weeks teliospores are present which measure (3.8–5.9) × (5.4–8.2)μ; they occur terminally, singly and occasionally in pairs. They increase in size and at 7 weeks are (4.9–7.6) × (5.4–10.8)μ, terminal, intercalary, single and in short chains of 2–3 spores. The spores usually have one or two bud-like structures that may represent either vestigial clamp connections or undeveloped sister teliospores.

Life history: Nuclear stains and gross morphological observations indicate the following life cycle. All strains are self-sporulating and mating has not been observed. A septate mycelium without clamp connections is produced from a single cell. The nucleus migrates from the terminal mycelial cell into the developing teliospore. The nucleus divides and one daughter nucleus goes into a lateral bud-like projection on the side of the teliospore, while the other remains in the spore.

During germination the nucleus migrates from the teliospore into the promycelium. The nucleus divides and the two resulting nuclei go toward opposite ends of the promycelium. A septum is laid down that separates each nucleus and the two nuclei divide again, followed by additional formation of septa resulting in a four-celled promycelium. Numerous sporidia can be produced from each cell by repeated mitoses of the four nuclei. The ploidy of the sporidia has not been determined.

Fermentation: Negative.

Assimilation of carbon compounds:

Glucose	+	Raffinose	— or + (weak latent)
Galactose	— or + (weak)	Melezitose	—
L-Sorbose	—	Inulin	—
Sucrose	— or + (weak latent)	Soluble starch	—
Maltose	— or + (weak latent)	D-Xylose	— or + (weak)
Cellobiose	—	L-Arabinose	—
Trehalose	— or + (weak latent)	D-Arabinose	—
Lactose	—	D-Ribose	—
Melibiose	—	L-Rhamnose	—

Ethanol	— or + (latent)	α-Methyl-D-glucoside	—
Glycerol	+ (sometimes weak)	Salicin	—
Erythritol	—	DL-Lactic acid	—
Ribitol	—	Succinic acid	—
Galactitol	—	Citric acid	—
D-Mannitol	— or + (weak latent)	*m*-Inositol	—
D-Glucitol	—		

Assimilation of potassium nitrate: Positive.
Growth in vitamin-free medium: Positive.
Growth at 17 C: Weak; at 19 C: Negative.
Starch formation: Negative.
Gelatin liquefaction: Positive.
Splitting of arbutin: Negative.
Acid production on chalk agar: Negative.
Growth on 50% (w/w) glucose yeast extract agar: Negative.
Splitting of urea: Positive.
Type culture: AR-372 (CBS-5942) isolated from sea water off Joinville Island.

This species is named after the region in which the 46 isolates were obtained.

The method of obtaining spore germination can be varied. 5 C is the most suitable temperature; at higher temperatures, particularly 12 C, large amounts of mucous polysaccharides are produced that interfere with the microscopic observations. The usual germination procedure involves growing the culture on corn meal agar, followed by soaking in distilled water and then transferring to aqueous agar. The specific time requirements for the first two steps have not been determined. The best results were obtained with a total growth-soak period of 3 months. There can be considerable variation in the duration of the individual steps; for example, growth on corn meal agar for two weeks followed by soaking for ten weeks or conversely, growth for ten weeks with soaking for two weeks. The spores then germinate within one week on aqueous agar.

Leucosporidium frigidum sp. n.

Imperfect stage: *Candida frigida* di Menna 1966.

Di Menna (1966*a*) originally described this species as *Candida frigida* on the basis of three isolates collected from two Antarctic soil samples at Scott Base (di Menna, 1966*b*). Her description included abundant formation of pseudomycelium; fermentation of glucose, sucrose, galactose, and raffinose (sometimes weak or negative), but no fermentation of lactose or maltose. Sugars assimilated were glucose, sucrose, lactose, and galactose, but not

maltose. A starch-like substance was produced on glucose peptone agar and on malt extract agar. None of the strains grew above 20 C or without an external source of vitamins and they did not liquefy gelatin.

Leucosporidium frigidum sp. n.

Hyphae e cellulis singulis, uninucleatae, sine septis nodosis. Teliosporae intercalares aut terminales, sphericae aut ovoideae, (4–7) × (4–8) μ, incrementum ad (5–7.5) × (5.5–11) μ, singulae aut brevicatenatae.

In extracto malti cellulae sunt singulae, binae aut catenatae, (3.2–8.1) × (3.8–13.5) μ; annulus et sedimentum formantur post unum mensem. In agaro farinae maïs mycelium verum cum septis sed non nodosis; blastosporae abundant. Teliosporae intercalares aut terminales, sphericae (5–8 μ in diametro), singulae, binae aut brevicatenatae.

Fermentatio: glucosum (lente), galactosum (lente et exigue), saccharum (lente), raffinosum (absens aut exigue et lente); maltosum et lactosum non fermentantur.

Glucosum, saccharum, cellobiosum, lactosum, raffinosum, ethanolum assimilantur, at non maltosum, melibiosum, amylum, L-rhamnosum, nec erythritolum.

Assimilatio kalii nitratis: praesens.

Ad crescentiam vitaminae externae necessariae sunt.

Summa temperatura aucta: 19 C.

Amylum formatur.

Typus: Cultura 5AI ex nive antartica isolata per di Menna, in CBS No. 5270, Delft, Hollandia.

Growth in malt extract at 12 C: After 3 days the cells are single, in pairs and short chains. Individual cells measure (3.2–8.1) × (3.8–13.5) μ; there is a slight ring and a sediment. After one month there is a thick myceliated ring and a heavy sediment.

Growth on malt agar at 12 C: After 3 days the cells are (2.2–5.4) × (3.8–13.5) μ. Cells are not capsulated. The colony is smooth, dull, cream-colored, the texture is soft. The border is entire with a mycelial fringe. There is true mycelium with single to numerous blastospores that are often in short branched chains. At one month teliospores are intercalary and terminal, single, in pairs or in short chains. The spores range in size from 5–8μ in diameter. Clamp connections are not produced on the mycelium.

Dalmau plate cultures on corn meal agar at 12 C: True, branched, septate mycelium with clusters of blastospores and branched pseudomycelium are present after one week. The teliospores (Fig. 9, p. 445) are both terminal and intercalary, single, in pairs, and short chains. After 3 weeks they are spheroidal to ovoidal, measuring (3.8–7.0) × (3.8–8.2) μ. After 9 weeks they have increased in size to (5.4–7.6) × (5.4–10.8) μ.

Life history: Mycelium and teliospores develop directly from a single cell without mating. Clamp connections are not produced. Germination of the teliospores occurs at 5 C by growing the culture on corn meal agar for five weeks, followed by soaking in distilled water for 7–12 weeks and then trans-

ferring to aqueous agar. Germination was then observed after 2 weeks on the agar. The two isolates examined had different types of promycelia; 5AI is 1-celled (Fig. 10, p. 445), P-8 is 2-celled (Fig. 11, p. 445). Sporidia are terminal on 1-celled promycelia, lateral or terminal on 2-celled promycelia.

Nuclear stains of isolate P-8 indicate that the organism is uninucleate. The nucleus migrates to the teliospore and subsequently into the promycelium. The nucleus divides and a transverse septum separates the two nuclei resulting in the two-celled promycelium. Sporidia are formed and the nucleus divides repeatedly to provide a nucleus for each new sporidium.

The two isolates examined did not mate with each other nor with the other species in the genus.

Fermentation:

Glucose	+ (slow to moderate)	Maltose	—
Galactose	+ (weak and slow)	Lactose	—
Sucrose	+ (slow to moderate)	Raffinose	+ (weak and slow) or —

Assimilation of carbon compounds:

Glucose	+	D-Ribose	—
Galactose	+	L-Rhamnose	—
L-Sorbose	+ (latent)	Ethanol	+
Sucrose	+	Glycerol	—
Maltose	—	Erythritol	—
Cellobiose	+	Ribitol	+
Trehalose	+ (sometimes latent)	Galactitol	— or + (weak latent)
Lactose	+	D-Mannitol	+
Melibiose	—	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	—
Melezitose	—	Salicin	+ (sometimes weak)
Inulin	—	DL-Lactic acid	—
Soluble starch	—	Succinic acid	+
D-Xylose	+	Citric acid	+
L-Arabinose	+	*m*-Inositol	— or + (weak)
D-Arabinose	+ (weak latent)		

Assimilation of potassium nitrate: Positive.

Growth in vitamin-free medium: Negative; vitamins required for growth: biotin and thiamine.

Growth at 17 C: Positive (weak); at 19 C: Negative.

Starch formation: Positive (pH-independent).

Gelatin liquefaction: Negative.

Splitting of arbutin: Positive.

Acid production on chalk agar: Negative.

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

Splitting of urea: Positive.

Type culture: 5AI (CBS–5270) collected by di Menna at Scott Base, Antarctica.

Leucosporidium gelidum sp. n.

Imperfect stage: *Candida gelida* di Menna 1966.

The original description of *C. gelida* (di Menna, 1966*a*) included well-developed pseudomycelium; fermentation of glucose, sucrose, maltose (may be weak or negative), galactose (may be weak) and raffinose (may be weak), but not of lactose. Sugars assimilated were glucose, sucrose, maltose, and galactose, but not lactose. Potassium nitrate was assimilated; a starch-like substance was produced on glucose peptone agar and on malt extract agar; none of the strains grew without an external vitamin source. All isolates liquefied gelatin.

Di Menna (1966*b*) obtained 23 isolates of this species from nine Antarctic soil samples collected at the Campbell-Mawson, Mawson-Koettlitz, Beardmore-Nimrod, and Shackelton-Axel Heiberg Glaciers and from Scott Base and twenty isolates from four Greenland soil samples near Mastersvig airstrip (72°N, 23°W, altitude 500 ft.). The common constituent of most of the samples was plant material such as mosses, lichens and algae.

Leucosporidium gelidum sp. n.

Hyphae e cellulis singulis uninucleatae, sine septis nodosis. Teliosporae intercalares aut terminales, subovoideae (3.8–5.4) × (4.6–6.5) μ, singulae aut binae.

In extracto malti cellulae sunt singulae aut binae (2.2–5.9) × (4.8–9.2) μ. Post unum mensem annulus et sedimentum formantur. In agaro farinae maïs mycelium verum cum septis et blastosporae sed non nodosis.

Fermentatio: Glucosum (lente), galactosum (exigue), maltosum (lente aut absens), saccharum, raffinosum (absens, interdum exigue), lactosum (absens).

Glucosum, galactosum, saccharum, maltosum, cellobiosum, melibiosum, raffinosum, amylum, L-rhamnosum, ethanolum (lente) assimilantur, at non lactosum nec erythritolum.

Kalium nitricum et natrium nitrosum assimilantur.

Ad crescentiam vitaminae externae necessariae sunt.

Summa temperatura aucta: 20 C.

Amylum formatur.

Typus: Cultura 2 AHIO ex humo antarctica isolata per di Menna, in CBS No. 5272, Delft, Hollandia.

Growth in malt extract at 12 C: After 3 days the cells are single and in pairs measuring (2.2–5.9) × (4.8–9.2) μ. A light sediment but no ring or film is formed. After one month there is a light sediment and a thick myceliated ring.

Growth on malt agar at 12 C: After 3 days the cells measure (2.2–5.9) × (3.8–13.5) μ. After one month the growth is soft, smooth, semi-glistening, and raised. The periphery is heavily myceliated, lobate and wrinkled. True, branched

mycelium with regularly occurring septa is present. Few blastospores are formed. There is an abundance of terminal and intercalary teliospores that are single, in pairs, and in short chains; they measure (5.4–10.8) × (6.5–13.5) μ.

Dalmau plate culture on corn meal agar at 12 C: After 3 days pseudomycelium and true, branched, septate mycelium with occasional clusters of blastospores are present, as well as small and poorly developed terminal and intercalary teliospores that are single and in pairs. After 3 weeks the spores are spheroidal to slightly ovoidal (3.8–4.6) × (4.6–5.9) μ.They do not increase much in size after 9 weeks of growth (3.8–5.4) × (4.6–6.5) μ.

Life history: A true mycelium develops from a single cell in the absence of mating. Teliospores form on the mycelium, clamp connections are not produced. This type of teliospore formation is typical of the self-sporulating phase.

Germination was rare, sporidia (Fig. 12) are formed terminally on a one- or

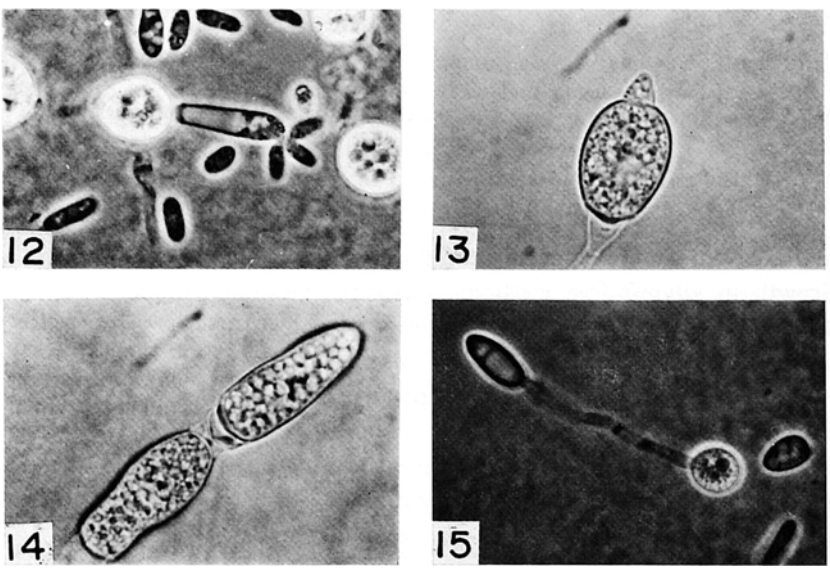


Fig. 12. *Leu. gelidum* teliospore with single-celled promycelium and terminal sporidia. Phase contrast, 940 ×.

Figs. 13 and 14. *Leu. nivalis* teliospores. Bright field, 940 ×.

Fig. 15. *Leu. stokesii* formation of a mycelium and teliospore (right) from a single cell (left). Phase contrast, 940 ×.

two-celled promycelium. In some cases sporidia appear to be produced on an extremely short promycelium.

The strain examined did not mate with isolates of any of the other species in the genus.

Fermentation:

Glucose	+ (slow)	Maltose	+ (slow) or —
Galactose	+ (weak)	Lactose	—
Sucrose	+	Raffinose	+ (weak) or —

Assimilation of carbon compounds:

Glucose	+	D-Ribose	— or + (latent)
Galactose	+	L-Rhamnose	+
L-Sorbose	+ (latent)	Ethanol	+ (latent)
Sucrose	+	Glycerol	—
Maltose	+	Erythritol	—
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	—
Lactose	—	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	— or + (latent)
Melezitose	+	Salicin	+
Inulin	—	DL-Lactic acid	—
Soluble starch	+	Succinic acid	+ (sometimes weak)
D-Xylose	+	Citric acid	+ (sometimes weak)
L-Arabinose	+	*m*-Inositol	— or + (latent)
D-Arabinose	— or + (latent)		

Assimilation of potassium nitrate: Positive.

Growth in vitamin-free medium: Negative; vitamins stimulating growth: biotin and thiamine.

Growth at 17 C: Positive; at 19 C: weak.

Starch formation: Positive (pH-independent).

Gelatin liquefaction: Positive.

Splitting of arbutin: Positive.

Acid production on chalk agar: Negative.

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

Splitting of urea: Positive.

Type culture: Strain 2AH1O (CBS 5272) isolated by di Menna from soil collected at Scott Base, Antarctica.

Two different methods of spore germination were employed at 12 C. In both cases spore germination was rare. (1) A young culture was inoculated on a malt extract agar plate and after one month transferred to aqueous agar. After 3 days germination was observed. (2) The one-month old malt extract agar culture was transferred to distilled water and soaked for four days, followed by transfer to aqueous agar. Germination was observed after one week on the aqueous agar.

Leucosporidium nivalis sp. n.

Imperfect stage: *Candida nivalis* di Menna 1966.

Di Menna (1966*b*) obtained fifty isolates from 6 Antarctic soil samples. One isolate came from each of the Campbell-Mawson and Nimrod Glaciers, 48 were from Scott Base. The original description (di Menna, 1966*a*) of *Candida nivalis* includes well developed pseudomycelium; fermentation of glucose, sucrose, galactose (may be weak or negative), raffinose (may be weak or negative), but not of maltose or lactose. Carbon compounds assimilated were glucose, sucrose, and galactose but not lactose or maltose. Potassium nitrate was assimilated; a starch-like substance was produced on malt extract agar and on glucose peptone agar; none of the strains grew without an external vitamin source. A small percentage of the strains liquefied gelatin.

Leucosporidium nivalis sp. n.

Hyphae e cellulis singulis, uninucleatae, sine septis nodosis. Teliosporae intercalares aut terminales, ovoideae aut longovoideae (4–11) × (4.5–16) μ, singulae, binae aut gregatae.

In extracto malti cellulae sunt singulae aut binae (3.2–7) × (4.9–9.2) μ. Post unum mensem annulus et sedimentum formantur; interdum velum aut insulae.

In agaro farinae maïs mycelium verum, pseudomycelium, blastosporae, blastoconidiae et teliosporae formantur.

Fermentatio: Glucosum (interdum lente), galactosum (exigue), saccharum, raffinosum (lente aut exigue), at non maltosum nec lactosum.

Glucosum, saccharum, cellobiosum, melibiosum, raffinosum, ethanolum assimilantur, at non maltosum, lactosum, amylum, L-rhamnosum, nec erythritolum.

Assimilatio kalii nitratis: praesens.

Ad crescentiam vitaminae externae necessariae sunt.

Summa temperatura aucta: 19 C.

Amylum formatur.

Typus: Cultura 2AH2 ex humo antarctica isolata per di Menna, in CBS No. 5266, Delft, Hollandia.

Growth in malt extract at 12 C: After 3 days the cells are ovoidal, single and in pairs. The individual cells measure (3.2–7.0) × (4.9–9.2) μ. There is a light sediment and ring, but no pellicle. After one month there is a heavy sediment, a moderate ring and in some cases a film or islets.

Growth on malt agar at 12 C: After 3 days the cells are ovoidal (2.7–6.5) × (3.2–12.4) μ. They occur singly and in pairs, they are not capsulated. After one month the growth is smooth, semi-glossy, soft, cream-colored, raised, and the border entire with tufts of hyphal development. The latter consists of true, branched, septate hyphae with blastoconidia as well as branched pseudomycelium. Teliospores are not abundantly produced; when found they are single, in pairs, and in short chains.

Dalmau plate cultures on corn meal agar at 12 C: After one week there is true mycelium with branched pseudomycelium and clusters of blastoconidia. Teliospores may be found after 3 weeks at 12 C but they are more abundant after 2–3 months. The 3 week old spores are spheroidal to ovoidal (3.8–5.4) × (4.9–6.5) μ, increasing in size, and by 9 weeks the spores are oval to elongate (3.8–10.8) × (4.6–16.2) μ. They are intercalary and occasionally terminal.

Life history: Mycelium develops from a single cell in the absence of mating. Teliospores (Figs. 13 and 14, p. 453) are intercalary and terminal on the mycelium. Clamp connections are not produced. Germination of the teliospores has not been observed.

The strain examined did not mate with isolates of any of the other species in the genus.

Fermentation:

Glucose	+ (sometimes slow and latent)	Maltose	—
Galactose	+ (weak)	Lactose	—
Sucrose	+	Raffinose	+ (latent or weak)

Assimilation of carbon compounds:

Glucose	+	D-Ribose	+ (latent)
Galactose	+	L-Rhamnose	—
L-Sorbose	+ (latent)	Ethanol	+
Sucrose	+	Glycerol	+ (latent)
Maltose	—	Erythritol	—
Cellobiose	+	Ribitol	+
Trehalose	+ (weak)	Galactitol	—
Lactose	—	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	—
Melezitose	—	Salicin	+
Inulin	—	DL-Lactic acid	—
Soluble Starch	—	Succinic acid	+ (weak and latent)
D-Xylose	+	Citric acid	— or + (weak)
L-Arabinose	+	*m*-Inositol	+ (sometimes latent)
D-Arabinose	+ (latent)		

Assimilation of potassium nitrate: Positive.

Growth in vitamin-free medium: Negative; vitamins stimulating growth: biotin, thiamine.

Growth at 17 C: Positive; at 19 C: very weak.

Starch formation: Positive on malt agar; negative in liquid glucose yeast nitrogen base.

Gelatin liquefaction: Weak.

Splitting of arbutin: Positive.

Acid production on chalk agar: Negative.

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

Splitting of urea: Positive.

Type culture: Strain 2AH2 (CBS–5266) isolated by di Menna from soil collected at Scott Base, Antarctica.

Leucosporidium stokesii sp. n.

Sinclair and Stokes (1965) examined the physiological properties of several yeasts isolated from Antarctic snow. Among the isolates studied was a strain labeled P-16 that Phaff, in the same publication, tentatively identified as a new species of *Candida*.

When we re-examined this isolate we found that it was a typical self-sporulating species, producing large numbers of teliospores. Because it differs physiologically from other members of the genus, we are describing P-16 as a new species. It is named after Dr. J. L. Stokes who isolated this strain and worked extensively in the area of psychrophilic yeasts.

Leucosporidium stokesii sp. n.

Hyphae e cellulis singulis uninucleatae, sine septis nodosis. Teliosporae abundant, intercalares aut terminales, singulae, binae, aut catenatae, ovoideae aut obpyriformae (3.2–5.4) × (4.9–8.1) μ.

In extracto malti cellulae sunt ovoideae (2.7–6.0) × (3.8–10.8)μ. Post unum mensem annulus et sedimentum formantur; interdum pellicula tenuis. In agaro farinae maïs mycelium verum ramosum, pseudomycelium, blastosporae gregariae et teliosporae formantur.

Fermentatio: Glucosum (lente), galactosum (lente, exigue), saccharum, maltosum (interdum exigue), raffinosum (interdum exigue), at non lactosum.

Glucosum, saccharum, maltosum, cellobiosum, raffinosum, amylum, L-rhamnosum, ethanolum assimilantur, at non lactosum, melibiosum, nec erythritolum.

Assimilatio kalii nitratis: praesens.

Ad crescentiam vitaminae externae necessariae sunt.

Summa temperatura aucta: 19 C.

Amylum formatur.

Typus: Cultura P–16, ex nive antarctica isolata per Sinclair et Stokes, in CBS No. 5917, Delft, Hollandia.

Growth in malt extract at 12 C: After 3 days the cells are (2.7–6.0) × (3.8–10.8) μ. There is a very light ring, no film, and a moderate sediment. After one month there is little development in the ring; a light film and a heavy sediment form.

Growth on malt agar at 12 C: After 3 days the cells are ovoidal to elongate (2.2–5.4) × (3.2–11.9) μ. After one month the streak culture is light cream-colored to whitish, with a raised surface that may be wrinkled or smooth with warty irregularities. The appearance is glistening and the texture soft. The edge

is undulate and heavily myceliated. The mycelium is true, branched and blastoconidia occur irregularly and sparsely along the mycelium. After one month teliospores are abundant, occurring singly and occasionally in pairs. Individual teliospores are (5.4–11.9) × (6.5–13.5) μ in size.

Dalmau plate culture on corn meal agar at 12 C: After 3 days there are true, branched, septate mycelium and pseudomycelium with abundant clusters of blastospores. After 3 weeks the teliospores present are oval to obpyriform (2.7–4.6) × (4.6–7.6) μ and usually terminal. After 9 weeks they have slightly increased in size to (3.2–5.4) × (4.9–8.1) μ; they occur mostly intercalary, single, in pairs, and in short chains. In general the teliospores are fewer in number and not as well developed as those found on malt agar.

Life history: Mycelium and teliospores develop directly from a single cell without mating (Fig. 15, p. 453). Clamp connections are not produced. Germination of the teliospores results in a short or long promycelium, which is one- to three-celled with lateral and terminal sporidia.

The strain examined did not mate with isolates of any of the other species in the genus.

Fermentation:

Glucose	+ (slow)	Maltose	+ (sometimes weak)
Galactose	+ (latent, weak)	Lactose	—
Sucrose	+	Raffinose	+ (sometimes weak)

Assimilation of carbon compounds:

Glucose	+	D-Ribose	+ (weak latent)
Galactose	+	L-Rhamnose	+
L-Sorbose	+ (latent)	Ethanol	+
Sucrose	+	Glycerol	—
Maltose	+	Erythritol	—
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	—
Lactose	—	D-Mannitol	+
Melibiose	—	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	—
Melezitose	+	Salicin	+
Inulin	—	DL-Lactic acid	—
Soluble starch	+ (sometimes weak)	Succinic acid	—
D-Xylose	+	Citric acid	—
L-Arabinose	+	*m*-Inositol	— or + (weak)
D-Arabinose	+ (weak latent)		

Assimilation of potassium nitrate: Positive.

Growth in vitamin-free medium: Negative; vitamins stimulating growth: biotin, thiamine.

Growth at 17 C: Positive; at 19 C: very weak.
Starch formation: Positive (pH-independent).
Gelatin liquefaction: Positive.
Splitting of arbutin: Positive.
Acid production on chalk agar: Negative.
Growth on 50% (w/w) glucose yeast extract agar: Negative.
Splitting of urea: Positive.
Type culture: Strain P-16 (CBS–5917) isolated from an Antarctic snow core sample by Sinclair and Stokes (1965).

Germination of the teliospores was not abundant, and the size of the promycelium varied with different methods. When the culture was grown on malt agar for 4 weeks, transferred to distilled water for 11 days, followed by transfer to aqueous agar, some of the spores germinated after 2 days and the promycelium was small with two large sporidia. However, when the spores were kept in distilled water for three months, the promycelia were long, one- to three-celled with lateral and terminal sporidia.

DISCUSSION

The species of *Leucosporidium* are candida-like organisms, which have a distinct budding yeast phase; most strains produce a pseudomycelium (rudimentary in a few instances) and form white to cream-colored colonies. The genus is, in many respects, similar to *Rhodosporidium*. The generic difference is based on the traditional separation of *Candida* and *Rhodotorula* by the presence or absence of carotenoid pigments. The phylogenetic validity of this method of separation with heterobasidiomycetous genera is subject to further study.

The life cycles of the species in both genera are similar to those reported for the Ustilaginaceae. However, a significant characteristic of the smut-fungi is their parasitic nature. In contrast host-parasite relationships have not been observed for species of *Leucosporidium* or *Rhodosporidium*. Their apparent relationship to the Ustilaginaceae invites the speculation that *Leucosporidium* and *Rhodosporidium* may be important in marine ecosystems as parasites of algae or higher plants.

The relationship to the Ustilaginaceae has influenced our choice of the terminology of the large, thick-walled, granular spores found in both genera. This type of spore is often termed a chlamydospore. According to Ainsworth (1961) a chlamydospore is "a thick-walled, non-deciduous, intercalary to terminal asexual spore made by the rounding up of a cell or cells". The term teliospore generally is relegated to the rusts and smuts, where the spores are

TABLE 1

Salient characteristics of the genus *Leucosporidium*

Compounds	*Leu. antarcticum*	*Leu. capsuligenum*	*Leu. frigidum*	*Leu. gelidum*	*Leu. nivalis*	*Leu. scottii*	*Leu. stokesii*
Fermentation:							
Glucose	–	L	+	+	+	–	+
Maltose	–	– or W	–	– or +	–	–	+
Sucrose	–	–	+	+	+	–	+
Raffinose	–	–	– or W	– or W	+	–	+
Assimilation:							
NO^3	+	–	+	+	+	+	+
Sucrose	– or WL	L	+	+	+	+	+
Maltose	– or WL	+	–	+	–	+	+
Cellobiose	–	L	+	+	+	+	+
Lactose	–	–	+	–	–	+ or –	–
Melibiose	–	–	–	+	+	–	–
Raffinose	– or WL		+	+	+	+	+
Melezitose	–	–	–	+	–	+	+
Rhamnose	–	–	–	+	–	+	+
m-inositol	–	+	– or W	– or L	+	–	+ or –

– = Negative reaction, + = Positive reaction, L = Latently positive reaction, W = Weakly positive reaction.

involved in sexual processes. Teliospores have been characterized by Fischer and Holton (1957) as "binucleate at first, finally uninucleate and diploid, and when they germinate the diploid nucleus undergoes a reduction division". This description of teliospores adequately describes the processes observed in *Rhodosporidium* (Banno, 1967) and in *Leu. scottii.*

Leucosporidium and *Rhodosporidium* are, in many respects, similar to the genus *Sporidiobolus* (Nyland, 1949). The formation of the dikaryotic mycelium and teliospores from a diploid cell in *Sporidiobolus* (Laffin and Cutter, 1959) appears to be the same as that found in the self-sporulating phase of *Rhodosp. toruloides* (Banno, 1967). However, the *Sporidiobolus* teliospores germinate to form ballistospores, a process that does not take place in *Rhodosporidium* or *Leucosporidium.*

Seven species are presently accepted in the genus. *Leu. scottii* and *Leu. capsuligenum* are separated by their inability to intermate and by their significant physiological differences (Table 1). Both species are heterothallic, but in the case of *Leu. capsuligenum* we have not observed the complete life cycle. The incomplete formation of teliospores suggests incompatible mating types or perhaps a nutritional deficiency. In contrast we have considerable information on the life cycle of *Leu. scottii*. The two basic unresolved questions are the ploidy of the self-sporulating strains and the genetics involved in the mating between self-sporulating and haploid strains.

The five self-sporulating species are separated by classical tests involving fermentation and assimilation of carbon compounds (Table 1). Until we understand the genetics involved in the self-sporulating life cycles, the validity of this taxonomic method will be open to question. For this reason we do not wish to consider *Candida* (*Leucosporidium*) *nivalis* and *Candida* (*Leucosporidium*) *frigida* as possible synonyms of *Candida curiosa* as suggested by Yarrow (1969). In the first place the strain of *C. curiosa* studied by us (CBS–5688) has not produced teliospores and can therefore not be included in *Leucosporidium* at this time. This strain is similar to *C. frigida* rather than to *C. nivalis*. In the second place the strains of *Leu. frigidum* and *Leu. nivalis* studied here could be separated satisfactorily on the basis of differences in lactose and melibiose assimilation.

This study was supported by the National Science Foundation through the Office of Antarctic Programs, Grant GA-3957. The authors are grateful to Dr. E. Marelli for editing the Latin diagnosis.

Received 21 *March* 1969

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