Species Status of *Hanseniaspora guilliermondii* Pijper

SALLY A. MEYER, RUTH E. BROWN, AND MAUDY T. SMITH

American Type Culture Collection, Rockville, Maryland 20852; and Centraalbureau voor Schimmelcultures, Laboratory of Microbiology, University of Technology, Delft, The Netherlands

Selected yeasts presently classified as *Hanseniaspora valbyensis* Kloecker 1912 were examined for their physiological and morphological properties and their deoxyribonucleic acid (DNA) relatedness. The strain designated *H. guilliermondii* Pijper 1928 is herein recognized as a species separate from *H. valbyensis*. *H. guilliermondii* is distinguished from *H. valbyensis* by insignificant DNA reassociations, assimilation of 2-keto-gluconate, growth at 37°C, and formation of four ascospores per ascus.

The genus *Hanseniaspora* and its imperfect counterpart *Kloeckera* were extensively studied by Miller and Phaff in 1958 (8). These authors recognized three species in the genus *Hanseniaspora*, namely *H. valbyensis*, *H. uvarum*, and *H. osmophila*. The species *H. guilliermondii* described by Pijper (10) and *H. melligeri* described by Lodder (3) were not examined by Miller and Phaff themselves, but were taken as synonyms of *H. valbyensis* on the authority of Lodder and Kreger-van Rij (5). A strain described by Castelli as *H. apuliensis* was placed in synonymy with *H. valbyensis* (8). Kreger-van Rij and Ahearn (2) in a study of the shape and structure of ascospores of *H. uvarum* and *H. valbyensis* stated that, among the synonyms of *H. valbyensis*, there is a distinct difference in maximum number of spores per ascus. The type strain *H. valbyensis* produces two hat-shaped ascospores, whereas *H. guilliermondii* and *H. melligeri* have four hat-shaped spores. Also, there is a difference in ability to grow at 37°C. Strains identical with the last two species can grow at 37°C, but strains with two spores can not. On the basis of these differences, Kreger-van Rij and Ahearn recognized that a separation of *H. guilliermondii* and *H. melligeri* from *H. valbyensis* might be justified.

The study of Nakase and Komagata (9) on the significance of deoxyribonucleic acid (DNA) base composition in the classification of the yeast genera *Hanseniaspora* and *Kloeckera* indicated that this suggestion was reasonable. A difference in guanine plus cytosine content between *H. valbyensis* and *H. guilliermondii*, being respectively 26.8 to 27.6 mol% and 31.0 to 31.7 mol%, was found. To determine the correct classification of *H. valbyensis* and *H. guilliermondii* (including *H. melligeri*), the physiological and morphological properties and the DNA relatedness were examined.

MATERIALS AND METHODS

Organisms and morphological and physiological characteristics. The 10 cultures examined are listed in Table 1. The physiological properties were investigated by the methods in reference 4. The assimilation tests were incubated on a shaker for 21 days. Ascosporeulation was induced by incubation of the organisms on 5% malt extract agar.

DNA base composition and DNA reassociation. DNA was isolated and purified by the methods previously described (7). DNA base composition (expressed as the mean molar percent of the guanine and cytosine content [mol% G+C]) was determined by the thermal denaturation method and formula [mol% G+C = $(T_m - 69.3)/0.41$] of Marmur and Doty (6). DNA from the type culture of *Candida parapsilosis* (ATCC 22019) was used as the standard DNA ($T_m = 85.9^\circ\text{C}$). The DNA-filter reassociation technique (1) was employed with modifications: DNA was extracted from cultures grown for 18 h (ATCC 10631) and 19 h (ATCC 10630) in 5% glucose in yeast nitrogen base (Difco) supplemented with 2 μCi of [6- ^3H]uracil per ml and 2 μCi of [8- ^3H]adenine per ml. DNA was sheared by passing the sample twice through a French mini-pressure cell at 20,000 lb/in². Vials containing one 1-cm filter with approximately 25 μg of immobilized single-stranded DNA were incubated with preincubation medium (PM) at 63°C for 2.5 h. The PM was removed, and 1.0 μg of fragmented, labeled DNA in 100 μl of 2 $\times$ SSC (SSC = 0.15 M sodium chloride and 0.015 M sodium citrate [pH 7.0 $\pm$ 0.2]) was added to each vial. Incubation was continued at 63°C in a reciprocal water bath shaker at approximately 20 strokes per min for 13 h. Filters were washed in 2 $\times$ SSC at 63°C and allowed to dry before the addition of scintillation fluid [Spectrafluor 2,5-diphenyloxazole-1,4-bis-(5-phenyloxazolyl)benzene (Amersham/Searle); prepared in toluene-Triton X-100 (2:1, vol/vol)].

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

RESULTS

The physiological characteristics of the yeasts and the number of spores per ascus permitted their separation into two groups (Table 2).

Three strains (CBS 480, CBS 481, and CBS 6618) were similar to the type strain (CBS 479) of *H. valbyensis*. They failed to grow at 37°C and on 2-keto-gluconate. Two hat-shaped ascospores were formed.

Five cultures (CBS 95, CBS 466, CBS 2567, CBS 4378, and CBS 6619) were identical with the type strain (CBS 465) of *H. guilliermondii*.

These five cultures include the type strain (CBS 466) of *H. melligeri*. They all grow at 37°C, utilize 2-keto-gluconate, and produce four hat-shaped spores.

The DNA base composition was determined for all 10 cultures (Table 3). The G+C content spanned the narrow range of 29.5 to 33.4 mol%. The four cultures of *H. valbyensis* had G + C values ranging from 29.5 to 30.0 mol%, whereas the six isolates grouped as *H. guilliermondii* exhibited a G+C content between 33.2 and 33.4 mol%. To determine further the extent of relatedness between these yeasts, DNA reassociation experiments were done (Table 4). A

TABLE 1. List of cultures of *H. valbyensis*

Designation ^a	Source
CBS 479, ATCC 10631	Isolated from soil in Denmark; type strain
CBS 95	From fermenting tomatoes
CBS 465, ATCC 10630	Isolated from diseased nail; type strain of <i>H. guilliermondii</i>
CBS 466	Isolated from dates; type strain of <i>H. melligeri</i>
CBS 480	Obtained from O. Winge, (Copenhagen)
CBS 481, ATCC 2108	Received from the ATCC via W. Lash Miller (Canada)
CBS 2567	Isolated from grape must
CBS 4378	Isolated from cecum of baboon
CBS 6618	Isolated from tomato, received from T. Nakase as AJ 4810
CBS 6619	Received from T. Nakase as AJ 5176

^a ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures.

TABLE 2. Salient characteristics to differentiate *H. valbyensis* and *H. guilliermondii*

Organism	No. of cultures tested ^a	2-Keto-gluconate	Growth at 37°C	No. of hat-shaped ascospores
<i>H. valbyensis</i>	4	—	—	2
<i>H. guilliermondii</i>	6	+	+	4

^a Type strains are included.

high degree of DNA reassociation (80 to 100%) was demonstrated between the DNA from the type strain of *H. valbyensis* and strains physiologically grouped as *H. valbyensis*; 100% DNA reassociation was revealed between the type strain of *H. guilliermondii* and isolates grouped as *H. guilliermondii*. No significant DNA reassociation was expressed between DNA from the type strain of *H. valbyensis* and the strains grouped as *H. guilliermondii*, or between DNA from the type strain of *H. guilliermondii* and the strains grouped as *H. valbyensis*.

DISCUSSION

The data, in particular DNA reassociation, presented in this study show clearly that the

TABLE 3. DNA base composition

Organism	$T_m^a \pm SD^b$	Mol% G+C
<i>H. valbyensis</i>		
ATCC 10631 ^c	81.6 $\pm$ 0.08	30.0
CBS 480	81.4 $\pm$ 0.13	29.5
CBS 481	81.4 $\pm$ 0.09	29.5
CBS 6618	81.4 $\pm$ 0.12	29.5
<i>H. guilliermondii</i>		
ATCC 10630 ^c	82.9 $\pm$ 0.16	33.2
CBS 2567	82.9 $\pm$ 0.10	33.2
CBS 4378	82.9 $\pm$ 0.11	33.2
CBS 6619	82.9 $\pm$ 0.10	33.2
CBS 95	83.0 $\pm$ 0.19	33.4
CBS 466	83.0 $\pm$ 0.14	33.4

^a Average of at least four thermal denaturation determinations.

^b SD, Standard deviation.

^c Type strain of species.

TABLE 4. DNA relatedness of *H. valbyensis* ATCC 10631 (CBS 479) and *H. guilliermondii* ATCC 10630 (CBS 465) to various strains of both species

Source of unlabeled DNA	<i>H. valbyensis</i> ATCC 10631 ^a	<i>H. guilliermondii</i> ATCC 10630 ^a
<i>H. valbyensis</i>		
ATCC 10631 ^b	(100)	15
CBS 481	100	16
CBS 6618	81	16
CBS 480	80	13
<i>H. guilliermondii</i>		
ATCC 10630 ^b	17	(100)
CBS 95	22	100
CBS 466	14	100
CBS 2567	23	100
CBS 4378	15	100
CBS 6619	10	100

^a Source of labeled DNA.

^b Type strain.

separation of *H. guilliermondii* from *H. valbyensis* as suggested by Kreger-van Rij and Ahearn (2) is sound. Since *H. melligeri* Lodder 1932 was described at a later date than *H. guilliermondii* (3), it is considered a synonym of this species. *H. apuliensis*, placed in synonymy with *H. valbyensis* by Miller and Phaff (8), was not examined in this study. Since the strain has lost its ability to sporulate, a phenomenon noted by many investigators (5, 8), *H. apuliensis* will be examined in a later study on the perfect-imperfect relationship of *Hanseniaspora* and *Kloeckera* species.

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REPRINT REQUESTS

Address reprint requests to: Dr. Sally A. Meyer, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

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