THE PHYLOGENETIC RELATIONSHIPS OF THE Q₆-EQUIPPED SPECIES IN THE TELEOMORPHIC APICULATE YEAST GENERA HANSENIASPORA, NADSONIA, AND SACCHAROMYCODES BASED ON THE PARTIAL SEQUENCES OF 18S AND 26S RIBOSOMAL RIBONUCLEIC ACIDS

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Eleven strains of species in the apiculate yeast genera Hanseniaspora, Nadsonia, and Saccharomycodes were examined for partial base sequence determinations of 18S and 26S rRNAs. In the partial base sequence of 26S rRNA (positions 493–622, 130 bases), percent similarities were 65–79, 76–84, and 70–76 between the genera Hanseniaspora and Nadsonia, the genera Hanseniaspora and Saccharomycodes, and the genera Nadsonia and Saccharomycodes, respectively. These apiculate yeasts showed 72–85 percent similarity with S. cerevisiae. In the partial base sequences of 26S rRNA (positions 1611–1835, 225 bases) and of 18S rRNA (positions 1451–1618, 168 bases), the number of base differences was calculated to be 38–26, 27–9, and 30–23, and 12–8, 11–5, and 13–8 between the above-mentioned genera, respectively. These apiculate yeasts showed 33–9, and 12–4 base differences, respectively, with S. cerevisiae. The three apiculate yeast genera were recognized phylogenetically based on the sequence data obtained. Some discussions were made, especially on dividing the members of the genus Hanseniaspora into two groups at the generic level.

The teleomorphic species of apiculate yeasts equipped with coenzyme Q-6 (Q₆ or Q₆) are currently classified in the following three genera: Hanseniaspora Zikes, NADSONIA, and SACCHAROMYCODES. For Part XLVIII, see ref. (21).
Nadsonia Sydow, and Saccharomycedes Hansen (10, 11, 13).

Saccharomycedes sinensis Yue (22) was once described as a second species of the genus Saccharomycedes characterized by diploid vegetative cells reproduced by bipolar budding on a very broad base (bud-fission), by the formation of spherical ascospores with smooth walls, and by showing a complex proton magnetic resonance (PMR) spectrum in the H-1 region (11, 14). Two species were recognized in the genus Nadsonia characterized by vegetative reproduction by bud-fission at both poles, by the formation of spherical brownish ascospores with spiny or warty walls, and by forming galactomannans in the cell walls (1, 4, 10).

The six species presently accepted in the genus Hanseniaspora have a common morphological character characterized by diploid vegetative cells reproduced by bipolar budding in basipetal succession (9, 13). However, the genus Hanseniaspora has a heterogeneous nature morphologically, serologically, and chemotaxonomically: it produces three types of ascospores, viz., either hat-shaped and released at maturity, spherical with an equatorial ledge, smooth or warty, and persistent, or spherical with warts and persistent (6, 9, 13) and includes two groups based on serological analyses, viz., Kloeckera Subgr... (I-) and Hanseniaspora Subgr... (IId) (15) and also two groups by PMR spectroscopy of mannose-containing polysaccharides, viz., the H'spora valbyensis and the H'spora osmophila groups (14).

This paper deals with partial base sequence determinations of Hanseniaspora, Nadsonia, and Saccharomycedes species, and discusses phylogenetic relationships among these apiculate yeast species based on the sequence data obtained.

MATERIALS AND METHODS

Yeast strains examined. Eleven strains of species in the genera Hanseniaspora, Nadsonia, and Saccharomycedes were examined for partial base sequence determinations of 18S and 26S RNAs (Table 1). These apiculate yeast strains were cultured as described previously (17).

Preparation of rRNAs. The rRNAs of the strains were prepared as described previously (16, 17).

Partial base sequencing of rRNAs. The rRNAs of the strains were partially sequenced by the method of Lane et al. (7) using reverse transcriptase (16, 17). The three DNA primers used in this experiment were the same as those described in previous papers (17, 20). The sequence data obtained were manually aligned. With respect to the sequence data in one region (positions 493 through 622, 130 bases) of 26S rRNA, percent similarity (=maximum homology) was calculated with Hitachi DNAsis, Ver. 7 (Hitachi Software Engineering Co., Yokohama, Japan) instead of calculating the number of base differences. In the other two regions (positions 1611 through 1835, 225 bases, of 26S rRNA and 1451 through 1618, 168 bases, of 18S rRNA), the number of base differences was calculated.

Coenzyme Q determinations. The coenzyme Q or ubiquinone (Co-Q) systems of H'spora occidentalis IFO 1819, N. commutata IFO 10029, and S'mycodes sinensis
Table. 1. Strains examined of *Hanseniaspora, Nadsonia,* and *Saccharomycodes* species.

<table>
<thead>
<tr>
<th>Species and strain</th>
<th>Other designation</th>
<th>mol% G + C of DNA*</th>
<th>Co-Q6</th>
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<tbody>
<tr>
<td><em>Hanseniaspora</em> Zikes</td>
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<tr>
<td><em>H</em>’spora guilliermondii Pijper</td>
<td>IFO 1411* CBS 465, A. Pijper</td>
<td>33.2, 31.0</td>
<td>Q-6</td>
</tr>
<tr>
<td><em>H</em>’spora occidentalis Smith</td>
<td>IFO 1819* CBS 2592, H. Kufferath</td>
<td>34.9</td>
<td>Q-6</td>
</tr>
<tr>
<td><em>H</em>’spora osmophila (Niehaus) Phaff, Miller et Shifrine</td>
<td>IFO 1753 K. Mikata, O-118m8, soil</td>
<td>33.9</td>
<td></td>
</tr>
<tr>
<td><em>H</em>’spora uvarum (Niehaus) Shehata, Mrak et Phaff</td>
<td>IFO 0630 CBS 5914, C. Rainbow</td>
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<td></td>
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<tr>
<td><em>H</em>’spora valbyensis Klöcker</td>
<td>IFO 1758 K. Mikata, D-3a13, flower</td>
<td></td>
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<tr>
<td><em>H</em>’spora vineae van der Walt et Tscheuschner</td>
<td>IFO 1415* CBS 2171, J. P. van der Walt</td>
<td>40.2, 37.8</td>
<td>Q-6</td>
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<tr>
<td><em>Nadsonia</em> Sydow</td>
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<td><em>N. commutata</em> Golubev</td>
<td>IFO 10029* CBS 6640, W. I. Golubev</td>
<td>39.5</td>
<td>Q-6</td>
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<tr>
<td><em>N. fulvescens</em> (Nadson et Konokotina) Sydow</td>
<td></td>
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<tr>
<td>var. elongata (Konokotina) Golubev</td>
<td>IFO 0665 NCYC 379, V. B. D. Skerman</td>
<td>Q-6</td>
<td></td>
</tr>
<tr>
<td>var. fulvescens</td>
<td>IFO 0666 NCYC 46, CL-1146</td>
<td>Q-6</td>
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<td><em>Saccharomycodes</em> Hansen</td>
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<tr>
<td><em>S</em>’mycodes ludwigii Hansen</td>
<td>IFO 0798* CBS 821</td>
<td>Q-6</td>
<td></td>
</tr>
<tr>
<td><em>S</em>’mycodes sinensis Yue</td>
<td>IFO 10111* CBS 7055, J. Yue, HS-506, soil</td>
<td>Q-6</td>
<td></td>
</tr>
</tbody>
</table>

* Data from Meyer et al. (9), Nakase and Komagata (12), and Golubev and Vagabova (3).
* Data from Yamada et al. (19).
* Data from the present experiment.

Abbreviations: *H*’spora, *Hanseniaspora; S*’mycodes, *Saccharomycodes; IFO, Institute for Fermentation, Osaka, Japan; CBS, Centraalbureau voor Schimmelcultures, Delft, the Netherlands; NCYC, National Collection of Yeast Cultures, Brewing Industry Research Foundation, Nutfield, England, U. K.; CL, Carlsberg Laboratorium, Copenhagen, Denmark.

IFO 10111 were determined as described previously (18).

Chemicals. The chemicals used in this experiment were those as described previously (17,20).

RESULTS

Partial base sequences in positions 493 through 622 rRNA

The primary partial base sequences of the examined strains of *Hanseniaspora, Nadsonia,* and *Saccharomycodes* species were aligned in positions 493 through 622 (2) (130 bases) of 26S rRNA (Fig. 1). Since base substitutions occurred at a high rate in this region, percent similarity was calculated in respective pairs of the strains by computer analysis using Hitachi DNAsis, Ver. 7.
As shown in Fig. 2, the percent similarities were 81–96 and 89–95 among the examined strains of the species within the genera *Hanseniaspora* and *Nadsonia*, respectively, and 76 between the strains of the species in the genus *Saccharomycodes*. There were relatively lower percent similarities (65–79, 76–84, and 70–76) between the genera *Hanseniaspora* and *Nadsonia*, between the genera *Hanseniaspora* and *Saccharomycodes*, and between the genera *Nadsonia* and *Saccharomycodes*. The percent similarities were calculated by computer analysis using Hitachi DNAsis, Ver. 7, in positions 493 through 622 (130 bases) of 26S rRNA. *Type strain.
spora and Saccharomyces, and between the genera Nadsonia and Saccharomyces, respectively. These apiculate yeast strains showed lower to higher percent similarities (72–85), compared with Saccharomyces cerevisiae IFO 2376.

Based on the calculated percent similarities, a dendrogram was drawn by the simple linkage method (5). As shown in Fig. 3, the two strains of H'spora guilliermondii and H'spora uvarum constituted a single cluster with 95 percent similarity. The strains of H'spora occidentalis, H'spora osmophila, and H'spora vineae constituted a separate cluster with 87–96 percent similarities. These two clusters were linked to one another at 85 percent similarity, in which H'spora valbyensis was included at 85 percent similarity. The strain of S'mycodes ludwigii was linked at 84 percent similarity to the Hanseniaspora clusters. On the other hand, the strain of S'mycodes sinensis was linked to S. cerevisiae IFO 2376 at 85 percent similarity, and then to the Hanseniaspora and the S'mycodes ludwigii clusters at 83 percent similarity. The Nadsonia cluster, in which the three strains of N. commutata, N. fulvescens var. fulvescens, and N. fulvescens var. elongata were located at 89–95 percent similarities, was linked at 79 percent similarity.

Partial base sequences in positions 1611 through 1835 of 26S rRNA

The primary partial base sequences of the strains examined of Hanseniaspora, Nadsonia, and Saccharomyces species were aligned in positions 1611 through 1835 (225 bases) of 26S rRNA (Fig. 4). The number of base differences was calculated among the strains examined.

As shown in Fig. 5, the examined strains of the species of the genus Hanseniaspora were divided into two groups based on the calculated number of base differences: 4–0 among those of H’spora guilliermondii, H’spora uvarum, and H’spora valbyensis, and 5–1 among those of H’spora occidentalis, H’spora osmophila, and H’spora vineae. It is noted that the number of base differences was large (24–
between the two groups. The strains examined of the species in the genus *Nadsonia* had 6-0 base differences. However, the base differences between the strains of the two species in the genus *Saccharomycodes* were not small but large (base differences, 17). The number of base differences was calculated to be 38-26, 27-9, and 30-23 between the genera *Hanseniaspora* and *Nadsonia*, between the genera *Hanseniaspora* and *Saccharomycodes*, and between the genera *Nadsonia* and *Saccharomycodes*, respectively. These apiculate yeast strains showed 33-9 base differences with *S. cerevisiae* IFO 2376.

Based on the calculated number of base differences, a dendrogram was drawn by the simple linkage method (5). As shown in Fig. 6, the strains of *H'spora*...
The strains of *H. guilliermondii* IFO 1411*, *H. uvarum* IFO 0830, and *H. valbyensis* IFO 1758 constituted a single cluster with 4–0 base differences. The strains of *H. occidentalis* IFO 1810* and *H. osmophila* IFO 1753 were linked to the latter cluster at 5–1 base differences. The strains of *S. ludwigii* IFO 0788* and *S. cerevisiae* IFO 2376 were linked to the latter cluster at 10–1 base differences. The strains of *S. sinensis* IFO 10111* was linked to the above-mentioned clusters at 15 base differences.

The number of base differences was calculated in positions 1611 through 1835 (225 bases) of 26S rRNA. *Type strain.

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**Fig. 5.** A triangle matrix based on the calculated number of base differences in the partial base sequences in positions 1611 through 1835 of 26S rRNA in strains of *Hanseniaspora*, *Nadsonia*, and *Saccharomycodes* species.

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**Fig. 6.** A dendrogram based on the calculated number of base differences in the partial base sequences in positions 1611 through 1835 of 26S rRNA in strains of *Hanseniaspora*, *Nadsonia*, and *Saccharomycodes* species.

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*Type strain.

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**guilliermondii**, *H. uvarum*, and *H. valbyensis* constituted a single cluster with 4–0 base differences. The strains of *H. occidentalis*, *H. osmophila*, and *H. vineae* constituted a separate cluster with 5–1 base differences. The strains of *S. ludwigii* and *S. cerevisiae* IFO 2376 were linked to the latter cluster at 9 base differences. These two clusters were linked to each other at 15 base differences. The strains of *S. sinensis* was at 16 base differences to the *Hanseniaspora* and the *Saccharomycodes* clusters. On the other hand, the *Nadsonia* cluster, in which the three strains of *N. commutata*, *N. fulvescens* var. *fulvescens*, and *N. fulvescens* var. *elongata* were located at 5–0 base differences, was linked at 23 base differences.
two species were not small but large (base differences, 7) in the genus Saccharomyces. The number of base differences was calculated to be 12–8, 11–5, and 13–8 between the genera Hanseniaspora and Nadsonia, between the genera Hanseniaspora and Saccharomyces, and between the genera Nadsonia and Saccharomyces, respectively. These apiculated yeast strains showed 12–4 base differences with S. cerevisiae IFO 2376.

Based on the calculated number of base differences, a dendrogram was drawn by the simple linkage method (5). As shown in Fig. 9, the strains of H'spora guilliermondii, H'spora uvarum, and H'spora valbyensis constituted a single cluster with 4–1 base differences. The strains of H'spora occidentalis, H'spora osmophila, and H'spora vineae constituted a separate cluster with 0 base difference. The strains of S'myces ludwigii and S'myces sinensis and S. cerevisiae IFO 2376 were linked.
to the former cluster at 5 base differences. These two clusters were linked to each other at 6 base differences. On the other hand, the Nadsonia cluster, in which the three strains of *N. commutata*, *N. fulvescens* var. *fulvescens*, and *N. fulvescens* var. *elongata* were located at 2 base differences, was linked at 8 base differences.

**Coenzyme Q systems of strains of apiculate yeast species**

The Co-Q systems of *H'spora occidentalis* IFO 1819, *N. commutata* IFO 10029, and *S'mycodes sinensis* IFO 10111 were found to be Q-6 (Table 1). The data obtained were identical with those of the other species of the three genera.

**DISCUSSION**

The Q<sub>6</sub>-equipped apiculate yeasts are currently classified in the three genera, *Hanseniaspora*, *Nadsonia*, and *Saccharomycodes*. The present experiment has demonstrated that the three genera are distinguished phylogenetically from each other (17).

Spencer and Gorin (14) reported that two species of the genus *Nadsonia* (= *N. fulvescens* var. *fulvescens* and *N. fulvescens* var. *elongata*) form galactomannans and are different in this respect from other mannan-forming apiculate yeast species. The two species examined in this experiment of the genus *Nadsonia* actually constituted a separate cluster phylogenetically distant from the other apiculate yeast species examined (percent similarity, 79; base differences, 23 and 8, respectively).

*Saccharomycodes sinensis* was found to be phylogenetically different from the other apiculate yeast species examined including the type species of the genus *Saccharomycodes*, *S'mycodes ludwigii* (percent similarities, 76–80; base differences 27–16 and 13–7, respectively). Accordingly, a separate genus can be set up for *S'mycodes sinensis* (17).

The six species examined of the genus *Hanseniaspora* were divided into two groups (or clusters) based on the sequence data obtained. A first group or cluster was composed of *H'spora guilliermondii*, *H'spora uvarum*, and *H'spora valbyensis*, and a second group or cluster was comprised of *H'spora occidentalis*, *H'spora osmophila*, and *H'spora vineae*. However, *H'spora valbyensis* (type species of genus *Hanseniaspora*) had somewhat distant phylogenetic relationships in the partial base sequence of 18S rRNA with the other two species (*H'spora guilliermondii* and *H'spora uvarum*) (base differences, 5–4). The first and the second groups or clusters mentioned above are characterized by a lower G+C content and a higher G+C content, respectively, in their DNA base compositions (9,12). The two groups or clusters are actually discriminated by ascospore morphology: mainly hat-shaped in the former and mainly warty round in the latter (6,9,13).

Tsuchiya et al. (15) serologically divided the members of the genus *Hanseniaspora* and their counterparts of the genus *Kloeckera* into two groups, viz., *Kloeckera Subgr*. . . (I–) (including *H'spora osmophila* and *H'spora vineae*) and *Hanseniaspora*
Subgr... (IId) (including \textit{H'spora valbyensis}, \textit{H'spora uvarum}, and \textit{H'spora guilliermondii}). Spencer and Gorin (14) classified the members of the genera \textit{Hanseniaspora} and \textit{Kloeckera} in two groups, viz., the \textit{H'spora valbyensis} and the \textit{H'spora osmophila} groups. \textit{Kloeckera javanica} (Klöcker) Janke, a supposed anamorph of \textit{H'spora occidentalis}, forms a mannan with the H-1 spectral region which resembles that of the former group in some respects and that of the latter group in others. The present authors' two groupings have been supported morphologically, serologically, and chemotaxonomically by the above-mentioned phenotypic features. Thus, the two groups or clusters recognized within the genus \textit{Hanseniaspora} should be distinguished from each other at the generic level (17).

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