The Phylogeny of Species of the Genus Issatchenkia KUDRIAVZEV (Saccharomycetaceae) Based on the Partial Sequences of 18S and 26S Ribosomal RNAs

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The ten strains of Issatchenkia species were examined for their partial base sequences of 18S and 26S rRNAs. In the 18S rRNA partial base sequences (positions 1451–1618, 168 bases), the strains of the species of Issatchenkia were found to be not uniform phylogenetically. The calculated base differences numbered 540. The strains of Issatchenkia species examined had 3-1 base differences with the type strain of Pichia membranaefaciens. Especially, the type strain of Issatchenkia orientalis, the type species of the genus Issatchenkia was found to be closely related phylogenetically to that of P. membranaefaciens. The calculated number of base differences was only one. The base sequences on the fingerprint segment were comprised of four bases (four kinds of AUAU, CCAU, AUAG, and ACAU), as found in P. membranaefaciens (ACAA). In the 26S rRNA partial base sequences, the calculated number of base differences was 8–0 (positions 1611–1835, 225 bases), and the calculated percent similarities were 61–80 (positions 493–622, 130 bases), within the genus Issatchenkia. Discussion was made phylogenetically and taxonomically, especially on the phylogenetic relationship between the type species of the genera Issatchenkia and Pichia and on a circumscription of the genus Issatchenkia.

Key words: Issatchenkia; Issatchenkia orientalis; partial base sequences of 18S and 26S rRNAs; phylogeny: taxonomy

The genus Issatchenkia KUDRIAVZEV was introduced with a single species, Issatchenkia orientalis KUDRIAVZEV. However, the name of the genus was not accepted for a long time. In 1980, Kurtzman et al. recognized the name of the genus Issatchenkia with an emendation. In “The Yeasts: A Taxonomic Study,” 3rd Edition, 1984, four species and two varieties are described: Issatchenkia occidentalis KURTZMAN, SMILEY et JOHNSON, I. orientalis, Issatchenka scutulata (PHAFF, MILLER et MIRANDA) KURTZMAN, SMILEY et JOHNSON var. scutulata, I. scutulata var. exigua (PHAFF, MILLER et MIRANDA) KURTZMAN, SMILEY et JOHNSON, and Issatchenka terricola (VAN DER WAL) KURTZMAN, SMILEY et JOHNSON. The members of the genus Issatchenkia are characterized morphologically by their warty, round ascospores1,3,4 and chemotaxonomically by their Q-7 system.3,4)

This paper describes the phylogenetic relationships of the Q8-equipped, warty, round ascospore-forming species of the genus Issatchenkia based on the partial base sequences of 18S and 26S rRNAs.

Materials and Methods

Yeast strains examined and cultivation. Ten strains of Issatchenkia species were examined for their partial base sequences of 18S and 26S rRNAs: I. occidentalis IFO 1904 (type strain, =CBS 5459, =NRRL Y-7552), I. orientalis IFO 1279 (type strain, =CBS 5147, =NRRL Y-5396) and IFO 1395 (=CBS 573, type strain of Candida krausei), I. scutulata var. exigua IFO 1896 (type strain, =CBS 6836, =NRRL Y-10920) and IFO 10077 [M. Mikata, single spore isolate of IFO 1896 (type strain), opposite mating type of IFO 1896 (1A), I. scutulata var. scutulata IFO 1895 (type strain, =CBS 6644, =NRRL Y-7663) and IFO 10081 [K. Mikata, single spore isolate of IFO 1895 (type strain), opposite mating type of IFO 1895 (3B)], I. terricola IFO 0933 (type strain, =CBS 2617, =NRRL YB-4310) and IFO 1888 (K. Mikata), and Issatchenkia species IFO 10088 (K. Mikata, Tw-33m7 from mushroom). These organisms were cultured as described previously.

Preparation and partial base sequencings of rRNAs and phylogenetic analyses. The preparation and isolation of rRNAs of these organisms were done as described previously.7 The 18S and 26S rRNAs prepared were partially sequenced as described in previous papers.8 The partial base sequences determined in 18S and 26S rRNAs were manually aligned. In positions 1451–1618, 168 bases, of 18S rRNA and positions 1611–1835, 225 bases, of 26S rRNA, the number of base differences was calculated in pairs of strains. However, the percent similarities (= maximum homologies) were calculated by computer analysis using a Hitachi DNAAsis (Ver. 7, Hitachi Software Engineering Co., Yokohama, Japan) in positions 493–622, 130 bases, of 26S rRNA, because of higher rates of base substitutions in this region. The phylogenetic trees of these organisms were constructed by the Neighbor-Joining method.11 The phylogenetic distance was defined by Kimura’s two parameters.12

Reagents and chemicals. The reagents and chemicals used in this experiment were the same as those described previously.7

Results and Discussion

The partial base sequences in the region of positions 1451–1618 (168 bases) of 18S rRNA of strains of Issatchenkia species are shown in Fig. 1a. The base sequences on the fingerprint segment7,13 (from position 1488) were comprised of four bases in all the strains examined of
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### Fig. 1. The Primary Partial Base Sequences of 18S and 26S rRNAs of Strains of *Isaticheniopsis* Species.

The primary partial base sequences of 18S (a) and 26S (b and c) rRNAs were aligned. The Arabic numerals indicate positions in *Saccharomyces cerevisiae*. N represents A, G, C, or U. The partial base sequences reported here will appear in the DDBJ Nucleotide Sequence Database under the accession numbers D89343-D89371. * Type strain.
Issatchenkia species: AUAU in the strains of *I. occidentalis*, CCAU in the strains of *I. orientalis*, AUG in the strains of *I. scutulata* var. *exigua* and *I. scutulata* var. *scutulata*, and *Issatchenkia* species IFO 10088, and ACAU in the strains of *I. terricola*. These four-based sequences are very similar to that (ACAA) of the type strain (IFO 10215) of *Pichia membranaefaciens*. However, the four kinds of sequences obtained here suggest that the genus *Issatchenkia* is not uniform phylogenetically.\(^7\)

The number of base differences was calculated in pairs of the strains examined. As shown in Fig. 2a, the strains of *Issatchenkia* species examined represented five to zero base differences within the genus, and the strains examined had 3-1 base differences from the type strain of *P. membranaefaciens* Hansen (IFO 10215). These data indicate that the genus *Issatchenkia* is not uniform phylogenetically in spite of comprising only four species and is close to the genus *Picha* Hansen. There were 12-10 base differences with that of *P. anomala* (Hansen) Kurtzman [≡ *Hansenula anomala* (Hansen) Sydow et Sydow] (IFO 10213)\(^{14}\) (data not shown). Especially, the type strain of the type species of the genus *Issatchenkia*, *I. orientalis* IFO 1279 was found to be very close to that of *P. membranaefaciens* (IFO 10215), the type species of the genus *Pichia*. The calculated number of base differences was only one.

Based on the partial base sequences determined, a phylogenetic tree was drawn by the Neighbor-Joining method.\(^{11}\) As shown in Fig. 3a, all the species of the genus *Issatchenkia* examined were phylogenetically divergent, when compared with the combination of *Williopsis saturnus* and *Saccharomyces cerevisiae* [base differences, three (not large)].\(^{16}\) In addition, *I. orientalis* was linked closely to *P. membranaefaciens*.

The partial base sequences in the region of positions 1611-1835\(^{10}\) (225 bases) of 26S rRNA of strains of *Issatchenkia* species are shown in Fig. 1b. The number of base differences was calculated in pairs of the strains examined. As shown in Fig. 2b, the strains examined of *Issatchenkia* species had eight to zero base differences. In contrast, there were 5-3 base differences with *P. membranaefaciens* IFO 10215, and 30-26 base differences with *P. anomala* IFO 10213\(^{14}\) (data not shown), respectively. Especially, *I. orientalis* IFO 1279 showed only three base differences from *P. membranaefaciens* IFO 10215.

Based on the partial base sequences determined, a phylogenetic tree was drawn by the Neighbor-Joining method.\(^{11}\) As shown in Fig. 3b, all the members of the genus *Issatchenkia* were located divergently, when compared with the combination of *W. saturnus* and *P. anomala* (base differences, four).\(^{16}\) *Issatchenkia orientalis* was located close to *P. membranaefaciens*.

The partial base sequences in the region of positions 493-622\(^{10}\) (130 bases) of 26S rRNA of strains of *Issatchenkia* species are shown in Fig. 1c. Since the rates of base substitutions are higher in this region, percent similarities were calculated in pairs of the strains examined by computer analysis using a Hitachi DNAsis (Ver. 7). *Issatchenkia orientalis* IFO 1395, the type strain of *C. krusei* had a somewhat different base sequence with 85 percent similarity from the type strain of this species, although the same partial base sequences were found in the other two regions examined of 18S and 26S rRNAs. As shown in Fig. 2c, the calculated percent similarities were 61-80 within the genus *Issatchenkia*. The strains of *Issatchenkia* species examined had 67-80 percent similarities with the type strain of *P. membranaefaciens* (IFO 10215), and 59-70 percent similarities with that of *P. anomala* (IFO 10213)\(^{14}\) (data not shown). However, *I. orientalis* IFO 1279, the type strain of the type species of the genus *Issatchenkia* was not so close to *P. membranaefaciens* IFO 10215, the type strain of the type species of the genus *Pichia*. The calculated percent similarity was 72 (not considered higher, but lower).

Based on the partial base sequences determined, a phylogenetic tree was drawn by the Neighbor-Joining method.\(^{11}\) As shown in Fig. 3c, all the species of the genus *Issatchenkia* were divergently distributed. *Issatchenkia orientalis* constituted a subcluster with *P. membranaefaciens*.

In 1980, Kurtzman et al.\(^9\) recognized the name of the genus *Issatchenkia* Kudriavzev with an emendation and transferred several species classified in the genus *Pichia* to the genus *Issatchenkia*. Our study demonstrated the genus *Issatchenkia* to be phylogenetically divergent, especially in the partial base sequences in the region of positions 493-622 (130 bases) of 26S rRNA. The calculated number of base
differences was 5-0 in the partial base sequencings (positions 1451-1618, 168 bases) of 18S rRNA and 8-0 in those (positions 1611-1835, 225 bases) of 26S rRNA (Fig. 2). However, the calculated percent similarities were 61-80 (very low to higher) in the partial base sequencings in the region of positions 493-622 (130 bases) of 26S rRNA.

We previously proposed a hypothesis on defining a circumscription of the yeast genera, that two taxons showing base differences of five or more can be distinguished from each other at the generic level in the sequence analyses in the region of positions 1451-1618 (168 bases) of 18S rRNA. According to our hypothesis or our opinion, either the genus Issatchenkia cannot be distinguished necessarily from the genus Pichia at the generic level, because the calculated number of base differences between the type species of both genera is only one, or cannot be divided necessarily into two or more genera, because the calculated number of base differences from the type species is three to four within the genus (Fig. 2a).

Peterson and Kurtzman (10) stated that the genus Issatchenkia is phylogenetically divergent, especially in I. terricola. In our sequence analyses, the type strain of I. terricola (IFO 0933) had six and three base differences from that of I. orientalis (IFO 1279) in the region of positions 1611-1835 (225 bases) of 26S rRNA and in the region of positions 1451-1618 (168 bases) of 18S rRNA, respectively. We reported that the ratio of the number of base differences in the region of positions 1611-1835 of 26S rRNA to that in

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**Fig. 3.**
the region of positions 1451–1618 of 18S rRNA is generally calculated to be two or more.19 The calculated ratio in the combination of *I. terecula* and *I. orientalis* is, in fact, two (six to three). The data obtained here indicate that *I. terecula* is not so distant phylogenetically from the type species of the genus *Issatchenkiia* (*I. orientalis*), as observed in the combination of *Ogataea minuta*17 (*= Pichia minuta*,

*Hansenula minuta* and *Ogataea glucozyma*17 (*= Pichia glucozyma*, *= Hansenula glucozyma*): the base differences are three and three (not so distant phylogenetically), respectively, in the combination of the two *Ogataea* species, and thus the calculated ratio is one but not two. However, the type strain of *I. terecula* gave a very low percent similarity comprised of 61 in the sequence analyses in the region of positions 493–622 (130 bases) of 26S rRNA, in contrast to the combination of *O. minuta* and *O. glucozyma* with 85 percent similarity.17

Peterson and Kurtzman18 constructed a phylogenetic tree on the basis of the total of the three partial base sequences determined of 18S and 25S (or 26S) rRNAs, so that the phylogenetic divergence stated in *I. terecula*18 is considered to be due to the partial base sequences found in the region of positions 409–613 (205 bases), where the base substitution rates are so high, of 25S (or 26S) rRNA.

Peterson and Kurtzman18 surprisingly did not analyze the partial base sequences of *P. membranaefaciens* as a reference standard. Our sequence analyses indicate that the type species of the genus *Issatchenkiia*, *I. orientalis* is closely related phylogenetically to that of the genus *Pichia* (*P. membranaefaciens*), with base differences of one and three, respectively, in the region of positions 1451–1618 (168 bases) of 18S rRNA and in the region of positions 1611–1835 (225 bases) of 26S rRNA, and with 72 percent similarity, which is not higher but lower, in the region of positions 493–622 (130 bases) of 26S rRNA.

According to Hendriks et al.20 and Wilmotte et al.21 *P. membranaefaciens* constitutes a cluster along with *I. orientalis*, the type species of the genus *Issatchenkiia*, in their phylogenetic trees based on the full sequence analyses of 18S rRNA. However, the two type species appear to be not very close phylogenetically: the distance between *I. orientalis* and *P. membranaefaciens* is almost identical with that between *Leucosporidium scottii* and *Sporobolomyces roseus*, an anamorph of the genus *Sporidiobolus*.21 A recent report of Kurtzman and Robnett22 pointed out that the two type species, in which the ascospore morphology is different, viz., hat-shaped ascospores in *P. membranaefaciens* and wart, round ascospores in *I. orientalis*, are very closely related phylogenetically to one another. However, they did not decide whether the two type species should be congeneric or not.

At the present time, the name of the genus *Issatchenkiia* is adequate to be retained, until the precise taxonomic position of *I. orientalis*, the type species of the genus *Issatchenkiia* is defined along with the other species of the genus, because ascospore morphology is known to be one of the most important criteria in yeast systematics.

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**References**
