Subgroup I contained 2 strains, ST-250 and ST-306. These strains had 34.8-34.9 mol% G+C. The sequence of D1/D2 domain of these two strains differed from type strain of *H. meyeri*, their closest species, by 20 (3.5%) and 22 (3.6%) nucleotides, respectively. ST-250 and ST-306 differed in 2 nucleotides including 1 gap between them. However, these two strains close to 3 undescribed yeasts. ST-250 and ST-306 differed from *H. uvarum* G10p1 and *H. meyeri* G4p4 in 3 and 6 nucleotides (including 1-5 gaps), respectively (Table 27). So that these strains are conspecific or sister species. Further, ST-250 and ST-306 are different species with *H. meyeri* G4p1 by 20-21 nucleotides including 6-7 gaps (Table 27). In the DNA-DNA hybridization, ST-250 and ST-306 showed the similarities of 20.8-23.6 % with the type strain of *H. meyeri* and 91.6% between them (Table 26). Subgroup I showed the similarities of 63.0-72.5 % and 58.4% with strains of subgroup II and subgroup III, respectively (Table 26). In the taxonomic criteria commonly employed, strains of subgroup I are differentiated from *H. meyeri* by ability to assimilate trehalose, sodium nitrate, growth at 36-37°C, inability to assimilate potassium nitrate and ethylamine and growth in 0.1% cycloheximide is negative. These data clearly suggested that ST-250 and ST-306 belonged to the same species and represented a new species of *Hanseniaspora*.

Subgroup II contained 3 strains, ST-464, ST-493 and ST-613. These strains had 34.9-35.3 mol% G+C (Table 26). They had identical sequences of D1/D2 domain and differed in 19 nucleotides from the type strain of *H. meyeri*, the nearest species (Table 27). However, subgroup II differed from undescribed yeasts of *H. uvarum* G10p1 and *H. meyeri* G4p4 in 2 and 4 nucleotides (including 1 and 2 gaps), respectively (Table 27). So that these strains are conspecific or sister species of subgroup II. Further, subgroup II is different species with *H. meyeri* G4p1 because 20 nucleotides including 7 gaps are different (Table 27). In the DNA-DNA hybridization, ST-464, ST-493 and ST-613 showed the similarities of 27.7-29.0% with type strain of *H. meyeri* (Table 26). In the taxonomic criteria commonly employed, subgroup II is differentiated from *H. meyeri* by ability to assimilate sodium nitrite, growth at 36-38°C, inability to assimilate potassium nitrate and ethylamine and growth in 0.1% cycloheximide is negative. These data clearly suggested that ST-464, ST-493 and ST-

613, are different species from known species and subgroup I, However, ascospore has not been found in this study so that strains in subgroup II will be described as new species of the genus *Kloeckera*.

Subgroup III contained a strain, ST-391. This strain had 35 mol %G+C. In the D1/D2 domain, ST-391 differed in 3-5 nucleotides from Subgroup I and Subgroup II (Table 27). This strain differed in 22 (3.9%) nucleotides including 2 gaps from the type strain of *H. meyeri*, the nearest species. ST-391 close to 3 undescribed yeasts by differed from *H. uvarum* G10p1 and *H. meyeri* G4p4 in 7 and 5 nucleotides (including 3 and 4 gaps), respectively (Table 27). Further, subgroup III are different from *H. meyeri* G4p1 by 24 nucleotides including 8 gaps (Table 27). In the DNA-DNA hybridization experiment, ST-391 showed similarity of 18.1% with the type strain of *H. meyeri* (Table 26). In the taxonomic criteria commonly employed, strains of ST-391 is differentiated from *H. meyeri* by ability to assimilate sodium nitrite, growth at 36-38°C, inability to assimilate glucono-δ-lactone, potassium nitrate and ethylamine and no growth in 0.1% cycloheximide. However, ascospore has not been found in this study so that strains in subgroup II will be described as new species of the genus *Kloeckera*.

Group III (ST-476)

ST-476 had a mol% G+C of 36.9 similar with the type strain of *H. clermontiae* (35.7-37.2%), the closest species. In the phylogenetic tree, this strain is distant from any known species of *Hanseniaspora* (Fig. 7). In D1/D1 domain, ST-476 differed in 10 nucleotides (1.7%) including 2 gaps from the type strain of *H. clermontiae*, the closest species, and more than 10 nucleotides from the type strains of related species (Table 28). In the taxonomic criteria commonly employed, strains of ST-476 is differentiated from *H. clermontiae* by ability to assimilate sodium nitrite, growth at 33-34°C, inability to assimilate potassium nitrate, ethylamine and no growth in 0.1% cycloheximide. Apparently, this strain represents a new species of *Kloeckera* because ascospore has not been found in this study.

Table 28 Number of nucleotide differences in D1/D2 domain among *Hanseniaspora/ Kloeckera* Group III and type strains of related species.

Group III	ST-476	*H. cle.*	*H. uva.*	*H. opu.*	CBS 8772	*H. mey.*	*H. lac.*
ST-476		10(2)	11	12(2)	12(2)	12(3)	14(3)
H. clermontiae	1.4		3	4	4	1	5
H. uvarum	1.9	0.6		5	5	4	6
H. opuntae	2.1	0.7	0.9		4	3	3
Hanseniaspora sp.CBS 8772	2.1	0.7	0.6	0.7		5	5
H. meyeri	2.1	0.2	0.8	0.6	0.9		6
H.lachancei	2.4	0.9	1.1	0.6	1.1	1.1	

Remark: A upper right triangle refers to nucleotide differences in D1/D2 including gaps. (Numerals in parenthes indicate the number of gaps) and lower triangle refers to % nucleotide differences.

Group IV (ST-484)

ST-484 had a mol% G+C of 36.1. In the phylogenetic tree, this strain was located at the cluster where the type strain of the closest species, *Kloeckera lindneri*, was located (Fig. 7). In the D1/D2 domain, ST-484 differed in 1 nucleotide from the type strain of *K. lindneri* and 7 nucleotides from the type strain of *H. valbyensis* (Table 29). The taxonomic criteria commonly employed of ST-484 is the same with *K. lindneri*. These data clearly suggests that ST-484 belongs to the species *Kloeckera lindneri*.