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Nucleotide Composition of Deoxyribonucleic Acid of Some Species of *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces*

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The buoyant density of deoxyribonucleic acid (DNA) from nine species and two varieties of *Cryptococcus*, three species and two varieties of *Rhodotorula*, and six species of *Sporobolomyces* was determined by CsCl density gradient equilibrium centrifugation. Several species were represented by two to four different strains. Expressed in moles per cent of guanine plus cytosine (GC content) the ranges were 49 to 65%, 52 to 70%, and 51 to 65% for *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces*, respectively. For each genus, the GC content was distributed into two discrete groups with averages ranging from 52 to 54 and 60 to 66, respectively. An analysis of these results suggested that the determination of GC content of DNA had a taxonomic value for these yeast genera.

It was previously reported (12) that the guanine plus cytosine (GC) content of deoxyribonucleic acid (DNA) among 13 species of yeast ranged from 38 to 63%. These GC values fell into two discrete groups with respective ranges of 38 to 48% and 49 to 63%. The group with lower values contained the seven ascosporogenous species analyzed. [*Candida pulcherrima* is now considered to belong to the ascosporogenous genus *Metschnikowia* (8).] The 15 species studied by Meyer and Phaff (6), all representing ascosporogenous species or their suspected asporogenous counterparts, had GC contents ranging from 34.9 to 48.3. They fit well, therefore, in the group with lower values, and the same can be said for 15 of the 18 *Candida* species investigated by Stenderup and Bak (11) and for a large number of sporogenous and some of the asporogenous yeasts analyzed by Nakase and Komagata (7). The other group (12) included two species of *Sporobolomyces* and one of each of the following genera: *Cryptococcus*, *Rhodotorula*, and *Torulopsis*. Values in the higher range have been reported for some species of *Candida* (7, 11), for some of *Torulopsis* and *Trichosporon*, for all species studied of *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* and for one species of *Tremella* (7).

The GC contents of DNA, from *Sporobolomyces salmonicolor* and *Rhodotorula mucilaginosa* of 63 and 61%, respectively, were at that time the highest ever reported (12) for fungi and were closer to those found for *Basidiomycetes* than for any other class of fungi. The similarity between the GC content of one species of *Rhodotorula* and one of the genus *Sporobolomyces* suggested an agreement with the hypothesis put forward by Lodder et al. (3) that some rhodotorulas are species of *Sporobolomyces* which have lost the ability to produce ballistospores. The genus *Sporobolomyces* appeared to be unique among fungi since it contained two species, namely, *Sporobolomyces roseus* and *S. salmonicolor*, whose DNA differed by 13% GC. Such a range was almost as wide as that found for fungal classes. These results suggested that an analysis of more species might very well have a taxonomic and phylogenetic value.

In the present work, the GC content of the DNA of a number of species of *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* was determined by CsCl density gradient equilibrium centrifugation. The results extend and confirm those described above.

MATERIALS AND METHODS

Organisms. All organisms came from the yeast collection of the Department of Food Science and Technology, University of California, Davis, California. Some cultures originated from the Centraal Bureau voor Schimmelcultures, Yeast Division, Delft, the Netherlands (CBS).

Growth and harvest. The organisms were grown in the medium of Bartnicki-Garcia and Nickerson (2) at 25 C on a rotary shaker in baffled, long-necked, 2-liter Erlenmeyer flasks and were harvested by centrifugation.

Extraction and purification of DNA. The cells were washed with a solution containing 0.1 M ethylenediaminetetraacetate and 0.15 M NaCl. The cells were ground in liquid N_2 and the DNA was extracted and purified as described elsewhere (13). When this procedure failed to yield DNA preparations with a molecular weight suitable for buoyant density analysis, it was replaced by that of Smith and Halvorson (10) except that an incubation with α-amylase (13) was included and isopropanol precipitation (4) was omitted. The two procedures were tried on some of the yeasts and yielded DNA preparations with identical buoyant density.

Determination of GC content. The method of Meselson, Stahl, and Vinograd (5) was used for the determination of the buoyant density of the DNA. The conversion into percentage GC was calculated according to Schildkraut, Marmur, and Doty (9). SP 8 bacteriophage DNA kindly supplied by M. Mandel (M. D. Anderson Hospital, Houston, Texas) was used as a reference. All DNA buoyant densities were related to that of *Escherichia coli*, which was taken to be 1.710 g/c^3 (9).

RESULTS

The GC contents of the DNA from two or more strains of most of the nine species of *Cryptococcus*, three of *Rhodotorula*, and six of *Sporobolomyces* were analyzed (Table 1). For each strain a minimum of two determinations was made on the same DNA preparation. Values for *S. salmonicolor* are similar to those reported earlier, but those for *S. roseus* were somewhat higher (12). The percentage GC in each of the three genera is distributed into two discrete groups of values which either do not overlap or overlap only slightly. As one can see, this dichotomy also exists within some species.

Some DNA preparations displayed in the CsCl gradient a minor band in addition to the main one. The buoyant density of this minor band was constant from one preparation of the same strain to another but it varied (from a buoyant density of 1.672 to 1.711 g/cm^3) from one strain to another. There was no overall correlation between the percentage GC of the major and minor DNA components, and there was also no indication that the percentage GC of these minor bands had a systematic value. This bimodal distribution of the DNA molecules has also been detected for many other species of fungi (Storck and Alexopoulos, *in preparation*). No attempt was made in the present work to determine the origin of these minor bands. However, on the basis of a detailed study of *Mucor subtilissimus* DNA, it appears by analogy that buoyant densities equivalent to less than 1.680 g/cm^3 (20% GC) might correspond to a "pseudo-satellite" composed of a polysaccharide contaminated with DNA (Moyer and Storck, *unpublished data*). Also, it was shown in a recent study (13) that fungal mitochondrial DNA had a GC content (calculated from buoyant density measurements) ranging from 28 to 44% for the

TABLE 1. *GC content in moles per cent of DNA from Cryptococcus, Rhodotorula, and Sporobolomyces species*[a]

Organism	GC content
Cryptococcus	
C. flavus CBS 331 syn. *Rhodotorula flava*	55.0
C. gastricus CBS 1927	51.0
C. gastricus CBS 2288	65.5
C. laurentii var. *magnus* CBS 569 syn. *Torula heveanensis*	49.0
C. laurentii var. *flavescens* FS 48-23A syn. *Rhodotorula peneaus*	58.0
C. melibiosum FS 52-87 syn. *Torulopsis melibiosum*	61.0
C. neoformans CBS 4572	51.5
C. skinneri FS 60-82	53.0
C. terreus CBS 1895	59.5
C. uniguttulatus CBS 1730 syn. *C. neoformans* var. *uniguttulatus*	51.5
C. uniguttulatus CBS 2994	58.0
Rhodotorula	
R. graminis CBS 2826	70.0
R. minuta var. *minuta* CBS 319	53.0
R. minuta var. *texensis* CBS 2177 syn. *R. texensis*	54.0
R. minuta var. *texensis* CBS 4407 syn. *R. tokyoensis*	52.5
R. pallida (CBS 320)	54.5
R. pallida (CBS 2623)	63.5
Sporobolomyces	
S. albo-rubescens CBS 482	63.0
S. holsaticus CBS 4029	62.0
S. holsaticus CBS 1522	64.0
S. holsaticus CBS 2630	65.0
S. holsaticus CBS 4209 syn. *S. coralliformis*	64.5
S. odorus CBS 483	65.0
S. pararoseus CBS 484	51.5
S. pararoseus 4217 syn. *S. marcillae*	55.0
S. pararoseus 2637	60.5
S. roseus CBS 486	56.0
S. roseus CBS 488 syn. *S. salmoneus*	55.5
S. roseus CBS 492 syn. *S. tenuis*	53.5
S. roseus CBS 1015	55.0
S. salmonicolor CBS 490	63.5
S. salmonicolor CBS 496	64.5

[a] Nomenclature used in this table is based on that employed by J. Lodder (*in press*). Abbreviations: CBS, Centraal Bureau voor Schimmelcultures; FS, Yeast Collection Food Science Department, University of California, Davis.

14 species which were analyzed. Since the majority of the satellite bands found in the DNA of the yeast analyzed in the present work had percentage GC within that range, it is suggested that they were of mitochondrial origin.

All the values listed in Table 1 were grouped and averaged; range and SE were calculated. These indexes are presented in Table 2. The overall distribution of percentages GC as well as the percentage for each genus were separated into two distinct groups in order to have about the same frequency in each group. The borderline between the two groups was therefore set in a somewhat arbitrary fashion. For each distribution, two discrete groups exist with different arithmetic averages. A "t" test for the consistency of these averages was performed, and in all cases the probability indicated a significant difference.

DISCUSSION

The GC contents of the DNA samples of the 32 strains investigated ranged from 49 to 70%. This range is larger than it is for each of the genera. With the possible exception of *C. gastricus*, (2 strains) it also appears that the compositional diversity of a genus is greater than that for a species. Although the DNA of strains of *R. minuta* and its varieties, that of *S. roseus*, *S. holsaticus* and of *S. salmonicolor* varies little with respect to its GC content, that of other species such as *C. gastricus*, the varieties of *C. laurentii*, *S. pararoseus*, and possibly that of *C. uniguttulatus* is subject to considerable variation. Since similar taxonomic criteria were used for the separation of all of the strains listed in Table 1, it may be assumed that in some instances these criteria are inadequate to detect specific or generic differences between strains. It remains that the range of GC content in the three yeast genera studied is on the average much larger than for other fungi (12). As the results presented here suggest, this situation results from the existence of two groups of GC content within each genus, characterized by averages which on the basis of a "t" test appear to differ significantly from each other. Recently, Nakase and Komagata (7) reported the results of analyses of the GC contents of 140 species of yeast and yeast-like fungi belonging to 26 different genera. It should be noted that the values for GC contents of type species reported by Nakase and Komagata (7) are usually 3 to 5 percentage points lower than those reported by other investigators. It would seem that these differences are due to procedural or technical differences in the experiments reported. These Japanese investigators found intrageneric variation of 10 to 18% for *Cryptococcus* (5 species) and *Rhodotorula* (11 species), respectively. Although their survey shows that such variations are not limited to these two genera, the average ranges were larger for the *Cryptococcaceae* than for the *Saccharomycetaceae*. This feature was also noted by Meyer and Phaff (6). In agreement with values previously reported (12) and those found in the present work, those presented by Nakase and Komagata (7) clearly demonstrate that, among the *Cryptococcaceae*, the yeasts belonging to *Cryptococcus* and *Rhodotorula* exhibited high GC contents, 46 to 56% and 47.5 to 65.5%, respectively. These authors found that organisms with strong urease activity had high GC contents without exception. This group in-

TABLE 2. *Statistical indexes of frequency distribution of GC content of DNA from Cryptococcus, Rhodotorula, and Sporobolomyces*[a]

Taxonomic group	No. of strains analyzed	Range	Avg	SE	"t" Value
All genera	32	49.4–70.0	58.1	5.48	33.90
%GC ≤ 56.0	16	49.0–56.0	53.2	1.89	
%GC ≥ 58.0	16	58.0–70.0	63.0	2.99	
Cryptococcus					
All strains	11	49.0–65.5	55.7	4.86	5.55
%GC ≤ 55.0	6	49.0–55.0	51.8	1.84	
%GC ≥ 58.0	5	58.0–65.5	60.4	2.78	
Rhodotorula					
All strains	6	52.5–70.0	57.9	6.56	6.33
%GC ≤ 54.5	4	52.5–54.5	53.5	0.25	
%GC ≥ 63.5	2	63.5–70.0	66.8		
Sporobolomyces					
All strains	15	51.5–65.0	59.9	4.71	35.20
%GC ≤ 56.0	6	51.5–56.0	54.4	1.51	
%GC ≥ 60.5	9	60.5–65.0	63.6	1.42	

[a] *P* for all genera and for *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* was ≪0.01.

cluded in addition to *Cryptococcus*, *Rhodotorula*, *Sporobolomyces*, *Torulopsis*, *Candida*, and *Trichosporon*, all genera which, as the authors point out, are supposed to be related to the *Heterobasidiomycetes*. Recently, Banno (1) obtained spore formation in mixed cultures of two strains of *Rhodotorula glutinis*, which has a DNA with 64% GC (7) and suggested that this genus is related to the *Ustilaginales*. In this respect, note that among the several hundred species of filamentous fungi which have been analyzed (Storck and Alexopoulos, *in preparation*), the average GC content of *Homobasidiomycetes* is higher than that of all other classes or subclasses.

In conclusion, the results of this work together with those of others clearly indicate that the GC content of DNA will become a useful taxonomic and phylogenetic criterion for yeasts and yeast-like fungi.

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LITERATURE CITED

1. Banno, I. 1967. Studies on the sexuality of *Rhodotorula*. J. Gen. Appl. Microbiol. 13:167–196.
2. Bartnicki-Garcia, S., and W. J. Nickerson. 1962. Nutrition, growth, and morphogenesis of *Mucor rouxii*. J. Bacteriol. 84:841–858.
3. Lodder, J., W. C. Slooff, and N. J. W. Kreger-Van Rij. 1958. The classification of yeasts, p. 1–62. *In* A. H. Cook (ed.), The chemistry and biology of yeasts. Academic Press Inc., New York.
4. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acids from microorganisms. J. Mol. Biol. 3:208–218.
5. Meselson, M., F. W. Stahl, and J. Vinograd. 1957. Equilibrium sedimentation of macromolecules in density gradients. Proc. Nat. Acad. Sci. U.S.A. 43:581–588.
6. Meyer, S. A., and H. J. Phaff. 1969. Deoxyribonucleic Acid Base Composition in Yeasts. J. Bacteriol. 97:52–56.
7. Nakase, T., and K. Komagata. 1968. Taxonomic significance of base composition of yeast DNA. J. Gen. Appl. Microbiol. 14:345–357.
8. Pitt, J., and M. W. Miller. 1968. Sporulation in *Candida pulcherrima*, *Candida reukaufii*, and *Chlamydozyma* species: their relationship with *Metschnikowia*. Mycologia 60:663–685.
9. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. 4:430–443.
10. Smith, D., and H. O. Halvorson. 1967. The Isolation of DNA from Yeast. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12, part A. Academic Press Inc., New York.
11. Stenderup, A., and A. L. Bak. 1968. Deoxyribonucleic acid base composition of some species within the genus *Candida*. J. Gen. Microbiol. 52:231–236.
12. Storck, R. 1966. Nucleotide composition of nucleic acids of fungi. II. Deoxyr bonucleic acids. J. Bacteriol. 91:227–230.
13. Villa, V. D., and R. Storck. 1968. Nucleotide composition of nuclear and mitochondrial deoxyribonucleic acid of fungi. J. Bacteriol. 96:184–190.