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Deoxyribonucleic Acid Base Composition in Yeasts

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The deoxyribonucleic acid base composition of 15 species of yeasts was determined to obtain further clues to or supporting evidence for their taxonomic position. Species examined belonged to the genera *Saccharomyces*, *Debaryomyces*, *Lodderomyces*, *Metschnikowia*, and *Candida*. The range of moles per cent guanine plus cytosine (GC content) for all yeasts examined extended from 34.9 to 48.3%. The sporogenous species and the asporogenous yeasts spanned the range with 36.6 to 48.3% GC and 34.9 to 48% GC, respectively. Three *Saccharomyces* species (*S. rosei* and related species) exhibited significantly higher GC contents than *S. cerevisiae*, whereas the fermentative species *D. globosus* revealed a %GC more aligned to the *S. rosei* group than to the nonfermentative *D. hansenii*. Similar GC contents were demonstrated by *L. elongasporus* and its proposed imperfect form *C. parapsilosis*. The range of GC contents of various strains of three *Metschnikowia* species studied was 6.1%, with the type strain of *M. pulcherrima* having the highest GC content (48.3%) of all of the yeasts examined.

The contribution of studies on deoxyribonucleic acid (DNA) base composition to the understanding of systematics and taxonomy of bacteria is well established (7). Similar information on other organisms is available only to a limited extent. Although Belozersky and Spirin (2), in their review of nucleic acids in microorganisms, suggested that an investigation of the DNA base composition of fungi from the viewpoint of speciation would be of significant value, only a few brief surveys (11, 16, 19, 20) have been made. These studies supported the usefulness of determining DNA base composition in fungal taxonomy.

In the most extensive study thus far reported, Storck (14) examined 30 species of fungi for their DNA base composition. Included in his investigation were representatives of the four major classes of fungi. For the first time in such a study, 14 species of yeast of 11 different genera were among the fungi examined. The four classes of fungi demonstrated a range of guanine plus cytosine (GC) contents of from 38 to 63%. The *Zygomycetes* (38 to 48%) and *Ascomycetes* (38 to 54%) were represented at the lower end of this range, whereas the *Deuteromycetes* (47 to 62%) and the *Basidiomycetes* (44 to 63%) were found at the upper level. *Hemiascomycetidae*, to which the ascosporogenous yeasts belong, were located in a narrower range of 39 to 45% GC.

Among the nonascosporogenous yeasts, the relatively high GC contents of a species of *Cryptococcus* (55%) and a species *Rhodotorula* (62%) were found to support previously suspected relationships to the lower basidiomycetes (1, 12), whereas others, *Torulopis stellata* and *Candida pulcherrima*, appeared to be related to the *Hemiascomycetidae*.

Recently, Stenderup and Bak (13) examined 18 species of *Candida* for their DNA base composition. The heterogeneity of the genus was expressed by the wide range of GC contents exhibited by these species. The variation extended over a range of from 34.9 to 57.6% GC. Those species with the lowest GC content (ca. 35%) were *C. tropicalis*, *C. claussenii*, *C. albicans*, and *C. stellatoidea*, whereas those with the highest GC content (ca. 54 to 57%) were *C. catenulata*, *C. brumptii*, and C. *zeylanoides*. Because of the very high GC content and the great compositional distribution of the DNA molecules of the last three species, the authors suggested that these organisms may have a phylogenetic origin different from the other *Candida* species studied.

MATERIALS AND METHODS

Organisms. All organisms used in this study are listed in Table 1. They were obtained from the yeast collection of the Department of Food Science and Technology, University of California, Davis (UCD). Some cultures originated from the Centraal Bureau voor Schimmelcultures (CBS), Delft, The Netherlands, or from the Northern Regional Research Laboratory Collection (NRRL), Peoria, Ill.

Growth conditions. All organisms were grown in a medium consisting of 0.5% yeast extract (Difco) and 5.0% glucose. The cultures (500 ml of medium in

1-liter Erlemeyer flasks) were incubated at room temperature on a rotary shaker and were allowed to grow for 36 to 48 hr. When large batches of yeasts were desired, an 80-liter fermentor (Stainless Steel Products Co., St. Paul, Minn.) was used to grow the organisms. A 45-liter amount of 0.5% yeast extract was sterilized in the fermentor, and 5 liters of 50% (w/v) glucose sterilized in distilled water was added. The inoculum consisted of 2 liters of a 48-hr starter culture (prepared on a shaker as described above). The cells were harvested in the late logarithmic phase of growth and were collected in a Sharples centrifuge. A minimal amount of saline ethylenediaminetetraacetate (0.1 M; EDTA) was added to the cells; the thick suspension was frozen and then dried in a Stokes freeze-dryer. The dried yeast was stored at room temperature in tightly closed bottles until needed.

Preparation of DNA. (i) For cell disruption, one part of dried yeast was suspended in four parts of saline EDTA (0.1 M), containing sodium dodecyl sulfate (2%, w/v) and mercaptoethanol (1%, v/v), and was placed in a 3-liter Fernbach flask to a depth of approximately 1 cm. The underside of the large cotton plug in the flask was soaked in chloroform, and the flask was incubated at 37 C for 16 to 20 hr.

(ii) For isolation and purification of DNA, the viscous suspension resulting from the above treatment was subjected to a modified method of Marmur (5). The modifications included several treatments with ribonuclease, the replacement of one-tenth the solvent volume by phenol in the deproteinization steps immediately following the treatments with ribonuclease, and dialysis of the final product against a standard saline citrate solution (SSC; 0.15 M NaCl–0.015 M trisodium citrate, *p*H 7.0 ± 0.2).

All DNA precipitates were collected by spooling around a glass rod.

Determination of GC content. The thermal denaturation temperature, T_m, of the DNA was determined in a solution of SSC by the method of Marmur and Doty (6), with a Zeiss spectrophotometer, a Haacke thermoregulator-constant temperature circulator, and a previously calibrated thermistor attached to a Tele-thermometer YSI model 42 SC. Marmur and Doty's (6) equation, $T_m = 69.3 + 0.41$ (G + C)%, was then used for the conversion of the T_m to GC%.

T_m determinations were made on DNA extracted from whole cells. It was assumed that the possible presence of mitochondrial DNA does not interfere with the determination of the T_m of nuclear DNA. The basis for this assumption is that mitochondrial DNA represents a very small percentage of the total DNA, and, in the *Ascomycetes* and *Basidiomycetes*, it melts at a temperature significantly lower than nuclear DNA (21).

RESULTS

The DNA base composition (moles % GC) of 15 species of yeast belonging to the genera *Saccharomyces*, *Debaryomyces*, *Lodderomyces*, *Metechnikowia*, and *Candida* was determined (Table 1).

A minimum of three independent determinations was made on the DNA obtained from a particular species. Two strains of *L. elongasporus* were included in order to determine the effect of strain variation. The type was isolated in California (10), whereas the other strain was isolated in South Africa (18). Both behaved similarly in all respects throughout the DNA purification procedure, and three independent T_m determinations revealed similar relative absorption curves with the average melting temperature of each strain varying only 0.1 C. Two independent DNA isolations were made of the same strain of *S. rosei* to determine variation imposed by technical manipulation. These two preparations revealed a difference of 0.2 C in the average of three T_m determinations.

The range of GC contents exhibited by all yeasts examined extended from 34.9 to 48.3%, whereas the range represented by species belonging to the *Hemiascomycetidae* was from 36.6 to 48.3%.

DISCUSSION

The range of GC contents of the *Hemiascomycetidae* is broader than the range of 39 to 45% as reported by Storck (14). Both lower and higher values were found. The DNA base composition of *Hemiascomycetidae* species included in this and other studies is compared in Table 2.

Debaryomyces hansenii and *S. cerevisiae* had the lowest GC content of all of the sporogenous yeasts included in our study. These two species were also examined by Storck (14). He found *D. kloeckeri* (now considered a synonym of *D. hansenii*) to have 40% GC, whereas *S. cerevisiae* had a GC content of 41%. It is unlikely that these higher values are due to strain variation, but probably are due to differences in equipment or experimental technique, or both. In the case of *S. cerevisiae*, our value of 37.1% is more in line with the values reported by other investigators (*see* Table 2).

The *Saccharomyces* species, *S. rosei*, whose type of ascus formation has at one time or other caused it to be classified in the genus *Torulaspora*, can be compared with *S. bisporus* and *S. inconspicuus*. These last two species, along with *S. rosei*, fall within a range of variation of 2.4% GC.

S. inconspicuus is physiologically similar to *S. rosei*, differing only in the lack of utilization of inulin and in having a variable sucrose and raffinose fermentation. *S. bisporus* is also physiologically similar to *S. rosei*. It differs by its inability to ferment inulin, trehalose, and raffinose. In addition. *S. bisporus* exhibits an iso-

gamous conjugation before ascus formation, whereas the other two species usually exhibit a type of mother-daughter cell conjugation. Recently, it was shown that the spores of *S. bisporus* are echinulate (n. Kreger-van Rij, *personal communication*). A study of the cell wall composition of various yeasts (M. Vidal-Leiria, M.A. Thesis, Univ. of California, Davis, 1967) indicated that the wall properties of *S. bisporus* are similar to those of *S. cerevisiae*, the type species of the genus; on this basis, *S. bisporus* may be considered a typical representative of the genus *Saccharomyces sensu strictu*. On the other hand, the base compositions of *S. bisporus*, *S. rosei*, and *S. inconspicuus* are considerably higher than that of *S. cerevisiae*. More typical species of *Saccharomyces* should be investigated to determine whether this difference in base ratio is significant.

D. globosus, a fermentative species, has in the past been aligned with *Torulaspora* species because of physiological similarities, in spite of its typical *Debaryomyces* characteristic of having rough-walled spores. It had a GC content similar to the *S. rosei* group of *Saccharomyces* and quite unlike the nonfermentative species *D. hansenii*, a typical member of *Debaryomyces*. The controversy concerning the proper taxonomic position of *D. globosus* is still unresolved. The sparse evidence, based on the GC content determined in this study, supports a relationship of *D. globosus* to the *Torulaspora*-like species of *Saccharomyces*. However, final decisions cannot be made until all of the species of *Debaryomyces* have been examined for DNA base composition.

The species belonging to the genus *Candida*, examined in this study, have numerous similar physiological properties and are included in the so-called *C. parapsilosis* group. This group (*sensu* Phaff and do Carmo Sousa; 8) was expanded by Fell and Meyer (3) to represent those *Candida* species that assimilate trehalose, xylose, and mannitol, but not lactose, melibiose, raffinose, dulcitol, inositol, or potassium nitrate.

A composite of species belonging to the *C. parapsilosis* group, examined thus far in this and other investigations, is shown in Table 3. A

TABLE 1. *DNA base composition of 15 species of yeast*

Organisms	UCD culture no.	Original source	T_m with standard deviation	GC%
Saccharomyces cerevisiae Hansen 1883	Bakers' yeast	Red Star Yeast Co.	84.5 ± 0.13	37.1
S. rosei (Guilliermond) Lodder et Kreger-van Rij 1913	C-450	(Etchells no. Y531)	87.3 ± 0.29	43.9
S. bisporus (Naganishi) Lodder et Kreger-van Rij 1917	62-24	CBS 702[a]	87.5 ± 0.09	44.4
S. inconspicuus van der Walt 1965	66-14	CBS 3003[a]	88.3 ± 0.22	46.3
Debaryomyces hansenii (Zopf) Lodder et Kreger-van Rij 1889	C-72	CBS	84.3 ± 0.26	36.6
D. globosus Klöcker 1909	68-37	CBS 764	87.8 ± 0.29	45.1
Lodderomyces elongasporus (Recca et Mrak) van der Walt 1952	53-57	CBS 2605[a]	85.6 ± 0.08	39.8
L. elongasporus (Recca et Mrak) van der Walt 1952	57-7	CBS 2606	85.5 ± 0.27	39.5
Metschnikowia reukaufii Pitt et Miller 1968	62-311[a]		86.6 ± 0.14	42.2
M. bicuspidata (Metschnikoff) Kamienski 1884	67-1	Miami no. 23-413	87.6 ± 0.05	44.6
M. pulcherrima Pitt et Miller 1968	C-214[a]		89.1 ± 0.21	48.3
M. pulcherrima Pitt et Miller 1968	64-13	NRRL-Y5941-53	86.7 ± 0.17	42.4
M. pulcherrima Pitt et Miller 1968	64-9	NRRL-YB-2272	87.6 ± 0.13	44.6
Candida tropicalis (Castellani) Berkhout 1910	60-31	Sawai	83.6 ± 0.28	34.9
C. parapsilosis (Ashford) Langeron et Talice 1928	61-27	CBS 604[a]	85.7 ± 0.24	40.0
C. diddensii (Phaff, Mrak et Williams) Fell et Meyer 1952	48-23H[a]		85.6 ± 0.22	39.8
C. atmosphaerica Santa Maria 1959	65-18	NRRL-Y-5979	86.2 ± 0.17	41.2
C. oregonensis Phaff et do Carmo Sousa 1962	60-73[a]		89.0 ± 0.03	48.0

[a] Type strain.

TABLE 2. *Comparison of GC%[a] of sporogenous yeasts as reported in the literature and determined in this study*

Species	GC%	Reference
Debaryomyces hansenii	36.6	
D. kloeckeri	40.0	14
D. globosus	45.1	
Saccharomyces rosei	43.9	
S. bisporus	44.4	
S. inconspicuus	46.3	
S. cerevisiae	37.1	
S. cerevisiae	37.0	6
S. cerevisiae	36.0	15
S. cerevisiae	38.0	11; Smith[c]
S. cerevisiae	41.0	14
S. lactis[b]	40.0	Smith
S. fragilis[b]	42.0	14
S. fragilis	41.0	11
S. dobzhanskii[b]	43.0	Smith
Metschnikowia reukaufii	42.2	
M. bicuspidata	44.6	
M. pulcherrima	48.3	
M. pulcherrima	44.6	
M. pulcherrima	42.4	
Lodderomyces elongasporus	39.8	
L. elongasporus	39.5	

[a] All values reported were obtained by T_m determination.

[b] Species now classified in the genus *Fabospora* according to Kudriavzev (4) or in the genus *Kluyveromyces* according to van der Walt (17).

[c] J. D. Smith, Ph.D. Thesis, Univ. of Wisconsin, Madison, 1967.

TABLE 3. *Comparison of GC%[a] of species of the Candida parapsilosis group as reported in the literature and determined in this study*

Species	GC%	Reference
Candida tropicalis	34.9[b]	
	34.9	13
C. claussenii	34.9	13
C. albicans	35.1	13
C. stellatoidea	35.7	13
C. parapsilosis	40.0[b]	
	40.8	13
C. diddensii	39.8[b]	
C. atmosphaerica	41.2[b]	
	39.8	13
C. pulcherrima[c]	48.0	13,14
C. oregonensis	48.0[b]	

[a] GC% determined by thermal denaturation method.

[b] GC% determined in this study.

[c] Now classified as *Metschnikowia pulcherrima*.

wide range of GC contents (34.9 to 48.0%) is evident. *C. tropicalis* has the lowest GC%; an identical value was obtained by Stenderup and Bak (13). This species differs from the rest of the *Candida* species listed in its ability to ferment both sucrose and maltose strongly. *C. claussenii*, *C. albicans*, and *C. stellatoidea*, three closely related species, possessed similar GC contents (13), and, together with *C. tropicalis*, these species have the lowest GC% within the *C. parasilosis* group (ca. 35%).

In the case of *C. atmosphaerica* and *C. parapsilosis*, Stenderup and Bak (13) obtained values close to those reported here. Of special interest is the %GC of *C. atmosphaerica* found by Stenderup and Bak; this value is the same as the value obtained in the present study for *C. diddensii*, but somewhat lower than that found for *C. atmosphaerica*. *C. atmosphaerica* was proposed as a synonym of *C. diddensii* by Fell and Meyer (3), who reported considerable strain variation in this ubiquitous species. Different extents of mycelium production, variability in the fermentation of glucose, and adaptation to the utilization of α-glucosides have been noted for numerous strains. Whether the small difference in GC content mentioned above is real and possibly related to these variable phenotypic expressions can only be answered by further investigations of additional strains.

C. oregonensis, with its relatively high GC content, is suspected of a small degree of relatedness to the other members of the *C. parapsilosis* group. However, it may possibly represent an upper limit, whereas other members of the group, yet to be examined, may occupy intermediate positions between *C. oregonensis* with 48.0% GC and those which fall close to 40% GC (*see* Table 3). Examination of the species *C. obtusa* and *C. solani* would be of particular interest, since they possess greater physiological similarity to *C. oregonensis* than the other members of the group. These three species represent the only galactose-negative members of the *C. parapsilosis* group.

C. parapsilosis contained the same GC content as *L. elongasporus*. Based on morphological and physiological characteristics, van der Walt (18) proposed that these two taxa represent the asexual and sexual form of one another. Verification of this supposition by mating experiments is precluded, since he found *L. elongasporus* to be homothallic.

Recently, *C. reukaufii* and *C. pulcherrima* were shown to produce needle-shaped ascospores under carefully defined conditions and, as a result, were transferred to the genus *Metschnikowia* (9). The GC content of *M. reukaufii* was 42.2%. The type strain of *M. pulcherrima* revealed a GC content of 48.3%, which is close to the values found for the type strain of *C. pulcherrima* (13) and for

another strain of this species (14). Two other strains of *M. pulcherrima* (*Chlamydozyma reukaufii* NRRL-Y-5941-53 and *C. pulcherrima* NRRL-YB-2272) demonstrated a lower GC content (*see* Tables 1 and 2). These last two strains were shown to form ascospores when mixed with their appropriate mating types. However, ascus formation was poor and morphological aberrations were evident (9). Possibly, chromosomal deletions or alterations have interfered with the mating ability of these strains and in turn have influenced the base composition of their DNA. Further examinations of other strains should help clarify this situation. *M. bicuspidata* [*sensu* Wickerham (22)] has a GC content intermediate between *M. reukaufii* and *M. pulcherrima*. Other species of this genus, such as *M. zobellii* and *M. krissii*, have not as yet been studied.

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LITERATURE CITED

1. Banno, I. 1967. Studies on the sexuality of *Rhodotorula*. J. Gen. Appl. Microbiol. 13:167–196.
2. Belozersky, A. N., and A. S. Spirin. 1960. Chemistry of the nucleic acids of microorganisms, p. 147–185. *In* E. Chargaff and J. N. Davidson (ed.), The nucleic acids, vol. 3. Academic Press, Inc., New York.
3. Fell, J. W., and S. A. Meyer. 1967. Systematics of yeast species in the *Candida parapsilosis* group. Mycopathol. Mycol. Appl. 32:177–193.
4. Kudriavzev, V. I. 1960. Die Systematik der Hefen. Akademie-Verlag, Berlin.
5. Marmur, J. 1961. A procedure for the isolation of DNA from microorganisms. J. Mol. Biol. 3:208–218.
6. Marmur, J., and P. Doty. 1962. Determination of the base composition of DNA from its thermal denaturation temperature. J. Mol. Biol. 5:109–118.
7. Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. Ann. Rev. Microbiol. 17: 329–372.
8. Phaff, H. J., and L. do Carmo Sousa. 1962. Four new species of yeast isolated from insect frass in bark of *Tsuga heterophylla* (Raf.) Sargent. Antonie van Leeuwenhoek J. Microbiol. Serol. 28:193–207.
9. Pitt, J. I., and M. W. Miller. 1968. Sporulation in *Candida pulcherrima*, *Candida reukaufii* and *Chlamydozyma* species: their relationship with *Metschnikowia*. Mycologia 60:663–685.
10. Recca, J. A., and E. M. Mrak. 1952. Yeasts occurring in citrus products. Food Technol. 6:450–454.
11. Rost, K., and H. Venner. 1964. Untersuchungen an Nucleinsäuren. X. Isolierung und Untersuchung von Deoxyribonucleinsäure aus Hefen. Z. Physiol. Chem. 339:230–237.
12. Slodki, M. E., L. J. Wickerham, and R. J. Bandoni. 1966. Extracellular heteropolysaccharides from *Cryptococcus* and *Tremella*: a possible taxonomic relationship. Can. J. Microbiol. 12:489–494.
13. Stenderup, A., and A. L. Bak. 1968. Deoxyribonucleic acid base composition of some species within the genus *Candida*. J. Gen. Microbiol. 52:231–236.
14. Storck, R. 1966. Nucleotide composition of nucleic acids of fungi. II. Deoxyribonucleic acids. J. Bacteriol. 91:227–230.
15. Tewari, K. K., W. Votsch, H. R. Mahler, and B. Mackler. 1966. Biochemical correlates of respiratory deficiency. VI. Mitochondrial DNA. J. Mol. Biol. 20:453–481.
16. Uryson, S. O., and A. N. Belozersky. 1960. Nucleotide composition of deoxyribonucleic and ribonucleic acids of certain fungi. Dokl. Akad. Nauk. SSSR Biochem. Sect. 132:117–119.
17. Van der Walt, J. P. 1965. The emendation of the genus *Kluyveromyces* v.d. Walt. Antonie van Leeuwenhoek J. Microbiol. Serol. 31:341–348.
18. Van der Walt, J. P. 1966. *Lodderomyces*, a new genus of the *Saccharomycetaceae*. Antonie van Leeuwenhoek J. Microbiol. Serol. 32:1–5.
19. Vanyushin, B. F., A. N. Belozersky, and S. L. Bogdanova. 1961. A comparative study of the nucleotide composition of ribonucleic acids and deoxyribonucleic acids in some fungi and myxomycetes. Dokl. Acad. Nauk. SSSR Biochem. Sect. 134:216–219.
20. Venner, H. 1963. Isolierung und Untersuchung von Nucleinsäuren aus Basidiomyceten. Z. Physiol. Chem. 333:5–19.
21. Villa, V. D., and R. Storck. 1968. Nucleotide composition of nuclear and mitochondrial deoxyribonucleic acid of fungi. J. Bacteriol. 96:184–190.
22. Wickerham, L. J. 1964. A preliminary report on a perfect family of exclusively protosexual yeasts. Mycologia 56:253–266.