JOURNAL OF BACTERIOLOGY, Jan., 1966
Vol. 91, No. 1

Printed in U.S.A.

Nucleotide Composition of Nucleic Acids of Fungi

II. Deoxyribonucleic Acids

ROGER STORCK

Department of Microbiology, The University of Texas, Austin, Texas

Received for publication 29 July 1965

ABSTRACT

STORCK, ROGER (The University of Texas, Austin). Nucleotide composition of nucleic acids from fungi. II. Deoxyribonucleic acids. J. Bacteriol. **91**:227-230. 1966.—The nucleotide composition of the deoxyribonucleic acids (DNA) present in extracts of 30 species of fungi was determined. The results were analyzed, together with those in the literature. It was found that the content, in moles per cent of guanine plus cytosine (GC content), varied from 38 to 63% in a distribution composed of 9 species of zygomycetes, 14 of ascomycetes, and 9 each of deuteromycetes and basidiomycetes. The GC content ranges were: 38 to 48% for the zygomycetes, 38 to 54% for the ascomycetes, 47 to 62% for the deuteromycetes, and 44 to 63% for the basidiomycetes. The GC content ranged from 38 to 40% for four *Mucor* species. The base composition of fungal DNA appears, therefore, to have a taxonomic and phylogenetic significance.

Knowledge of the nucleotide composition of deoxyribonucleic acid (DNA) has proven to be useful for the taxonomy of microorganisms. This is specially well demonstrated in the case of bacteria where several hundreds of species have already been analyzed (9).

In the instance of fungi, little information is available. The only exploratory work of some scope was performed by Uryson and Belozersky (16) and by Vanyushin, Belozersky, and Bogdanova (17). These authors analyzed two species of myxomycetes, two phycomycetes, four ascomycetes, two deuteromycetes, and four basidiomycetes, and found that the compositional diversity of DNA, when expressed in moles per cent of guanine plus cytosine (GC content), extended from 34 to 57%. DNA with less than 50% GC was found almost exclusively in myxomycetes and phycomycetes. The GC content was very close to 50% for the ascomycetes and significantly higher than 50% for the basidiomycetes. These few results led the Russian workers to the conclusion that the knowledge of DNA base composition could be used in studies on the systematics and phylogeny of the fungi.

The small number of analyses performed in other laboratories have confirmed the results obtained by Belozersky's group. Indeed, Venner (18) found 57 and 58% GC, respectively, for *Polyporus versicolor* and *Amanita strobiliformis* (basidiomycetes), and Minagawa, Wagner, and Strauss (11) discovered that the DNA of *Neurospora crassa* contained 54% GC. In *Saccharomyces cerevisiae*, however, DNA has a low GC content (36% GC), as shown many years ago by Zamenhof and Chargaff (19). More recently, Rost and Venner (12) obtained 41 and 42% GC, respectively, for *Saccharomyces fragilis* and *Schizosaccharomyces pombe*. From these results, it would appear that perhaps the GC content of the DNA from the Hemiascomycetidae is significantly lower than that of the DNA from the Euascomycetidae.

In the present work, 23 additional species belonging to four classes of Eumycotina have been analyzed. The results are in agreement with those cited above, and they clearly establish that the base composition of DNA has a systematic and phylogenetic significance for this group of eucaryotic microorganisms. In addition, fungal species having a significant difference between the base compositions of their homologous DNA and total ribonucleic acids (RNA) were found which may be useful for studies in morphogenesis.

MATERIALS AND METHODS

Organisms. Organisms were selected so that there would be about equal numbers of representatives of each group of fungi, including yeast as well as filamentous forms, and also species already analyzed by others. In each group, from two to four species be-

longing to the same genus were used to determine the degree of similarity of their DNA composition.

Growth and harvest. All organisms were grown and harvested as described previously (15), but the pellets and mycelial mats were washed twice with 0.15 M NaCl and 0.1 M ethylenediaminetetraacetate (EDTA), *p*H 8.0. The cells were ground with washed, 50-mesh sand in a chilled mortar.

Preparation of DNA. DNA was routinely prepared according to Marmur (7). Sometimes, however, the procedure devised by Cheng and Sueoka (4) for the extraction of crab muscle DNA was employed when the preparation was to be analyzed only by CsCl density gradient equilibrium centrifugation. In some cases, DNA precipitates could not be spooled around a glass rod and had to be collected by centrifugation. It was found that the molecular weight of fungal DNA varied greatly from one organism to another and from preparation to preparation, probably as a result of poor reproducibility of the grinding process. Estimates of molecular weights by sedimentation analyses (5), however, consistently yielded values higher than 10^6.

After the last purification step, the DNA was dissolved in 0.15 M NaCl plus 0.015 M trisodium citrate (SSC), *p*H 7.0. For melting experiments, the DNA preparations were always dialyzed against 1,000 volumes of SSC for 24 hr at 4 C; undialyzed and dialyzed preparations were stored in the frozen state.

Determination of GC content. The method of Meselson, Stahl, and Vinograd (10) was used for the determination of the buoyant density of the DNA sample, which was converted into GC content by employing the equation of Schildkraut, Marmur, and Doty (13). The following buoyant density values (in grams, per cubic centimeter) were used as references: 1.710 (*Escherichia coli*), 1.743 (SP 8 bacteriophage), 1.721 (*Pseudomonas fluorescens*), and 1.690 (*Spirillum linum*). The density of DNA solutions prior to centrifugation was adjusted as described by Baldwin and Shooter (1). Melting curves were obtained with a Gilford model 2000 multiple sample absorbance recorder. The temperature (T_m) corresponding to the midpoint of transition from double helix to random coil was determined according to Marmur and Doty (8), and their equation was used for the conversion of T_m values into GC content.

RESULTS

The results of our determinations, together with those of other investigators, are shown in Table 1. As can be seen, in most cases the GC content was determined from both buoyant-density and melting-temperature measurements, and the values were in good agreement. The greatest discrepancy was equal to 5% GC in the case of *Zygorhynchus moelleri*. Otherwise, the average difference between the two types of values was close to 1%. In several instances, the same orga-

TABLE 1. *Guanine plus cytosine (GC) content of DNA from fungi*

Organism	GC content (moles per cent)			Reference
	Buoyant density	T_m	C*	
Zygomycetes				
Absidia glauca	44	48		
Lichtheimia sp. (*Absidia*)			39	17
Mucor racemosus (1608)†	38			
M. rouxianus (4855)	38	41		
M. rouxianus (8097)	39			
M. rouxii (1894)	39			
M. subtilissimus (1743)	39	39		
M. subtilissimus (1909)	39	40		
Phycomyces blakesleeanus (−)	43	44		
			39	16
P. blakesleeanus (+)			39	16
Zygorhynchus moelleri	35	40		
Syncephalastrum racemosum	48	47		
Deuteromycetes				
Aspergillus niger	52	52		
Penicillium chrysogenum	52	52		
P. notatum	53	51		
Candida pulcherrima	46	48		
Cryptococcus albidus	55	55		
Rhodotorula mucilaginosa	61	63		
Torulopsis stellata	50	48		
Trichothecium roseum			50	17
Botrytis cinerea			50	17
Ascomycetes				
Dipodascus uninucleatus	43			
Endomyces reesii	39	41		
Debaryomyces kloeckeri	40	40		
Pichia membranefaciens	46	44		
Saccharomyces cerevisiae	40	41		
			36	19
	39			13
		38		12
S. fragilis	42	42		
Schizosaccharomyces octosporus	40	42		
S. pombe		42		12
Neurospora crassa	52	55		
	54			13
			54	11
Gelasinospora calospora	55	53		
Claviceps purpurea			53	17
Sporormia sp.	51	51		
Helvella esculenta			50	17
Sclerotinia libertiana			46	16
Basidiomycetes				
Sporobolomyces roseus	50	50		
S. salmonicolor	63	63		
Schizophyllum commune	58	58		
			57	17
Polyporus versicolor			57	18
Amanita strobiliformis			58	18
A. muscaria			57	17
Agaricus bisporus			44	16
Psalliota campestris			44	3
Bovista sp.			51	17

* C = chemical determination.

† Numbers in parentheses following *Mucor* species refer to Northern Regional Research Laboratory (Peoria, Ill.).

TABLE 2. *Distribution of GC content of DNA among fungi**

Taxonomic group	No. of species analyzed	Range	Avg
All classes	41	38–63 (25)	48.3
Zygomycetes	9	38–48 (10)	40.9
Ascomycetes	14	38–54 (16)	45.3
Basidiomycetes	9	44–63 (19)	54.6
Deuteromycetes	9	47–62 (15)	52.2
Mucor	4	38–40 (2)	39.1
Hemiascomycetidae	8	39–45 (6)	40.9
Euascomycetidae	6	46–54 (8)	49.7

* The range and average values are expressed in moles per cent GC.

nism was analyzed in two or more different laboratories by various methods. Again, an inspection of Table 1 (especially the case of *Saccharomyces cerevisiae* and *Neurospora crassa*) indicates a good agreement between these independent values. By both methods used in the present work, it was found that the GC content for a given DNA sample did not vary by more than 2% from one determination to another. In most instances, the distribution of the DNA molecules was unimodal at equilibrium in the CsCl gradients. However, in several instances, a satellite banding was found at a position corresponding to an average of 1.675 ± 0.002 g/cm^{-3}. It was demonstrated with *Mucor subtilissimus* that this material corresponded to a nucleic acid—polysaccharide complex which could be dissociated only by gel filtration and methylated albumin kieselguhr chromatography (Moyer and Storck, *in preparation*). It was also shown, however, that the presence of this satellite did not interfere with the position of the main DNA in the CsCl gradients at equilibrium, or with the T_m determinations.

For each organism listed in Table 1, the two or more values indicated were averaged and grouped in Table 2 according to taxonomic position. In the case of *Mucor* species, six strains belonging to four species were analyzed (Table 1). In total, 41 different species of fungi are included in this survey.

DISCUSSION

The GC content of fungal DNA ranges from 38 to 63%. This range is larger than it is for each class. The compositional diversity also decreases from classes and subclasses to genera and species. For example, in the Mucorales, the overall range of 8% (46 to 38%) decreases to 2% for the four *Mucor* species analyzed. However, with *Sporobolomyces*, a difference of 13% GC is found between two species. The 63 and 62% GC contents of DNA, respectively, from *Sporobolomyces salmonicolor* and *Rhodotorula mucilaginosa* are the highest ever reported for fungi. It is interesting to note that Lodder, Slooff, and Kreger-Van Rij (6) suggested that *Rhodotorula* species might originate from *Sporobolomyces* species having lost the ability to produce ballistospores. This case constitutes a good example of the possible use of the GC content of DNA to solve taxonomic problems, but more species need to be analyzed, especially since the DNA of *Sporobolomyces roseus* contains only 50% GC.

As shown in Table 2, the distributions of GC content for the taxonomic groups overlap, often by a single value, and, therefore, more are required to determine with confidence the range characteristic for each of these groups. In the meantime, however, it is possible to note the dominant features emerging from the present survey. On the average, the lowest GC contents are found in the zygomycetes and the highest ones in the basidiomycetes. Among the ascomycetes, GC contents lower than 50% are found (with the exception of *Sclerotinia libertiana*) exclusively for Hemiascomycetidae, for which the range in GC content is similar to that for the zygomycetes. In contradistinction, the GC content for the Euascomycetidae ranges from 46 to 54%, and, if *S. libertiana* is excluded, the range now extends from 50 to 54%. This situation suggests not only a sharp division of the ascomycetes, but also a possible link between zygomycetes and ascomycetes. It is interesting in this respect to note that *Dipodascus uninucleatus*, which is considered by many mycologists to be an intermediate between these two taxonomic groups, has 43% GC. The ranges for the deuteromycetes and the basidiomycetes are almost the same. However, if *Rhodotorula mucilaginosa* is ignored, the range for the deuteromycetes extends only from 47 to 55%, and is similar to that for the Euascomycetidae, to which most of the deuteromycetes are thought to be related. In conclusion, it appears that a thorough and critical analysis of the base composition of DNA will have a taxonomic and phylogenetic value for the fungi.

Belozersky and Spirin (2) found a weak positive linear correlation between the GC contents of homologous RNA and DNA, which was due, as shown by Spiegelman (14), to informational or messenger RNA present in small amounts in total RNA. It therefore became of interest to determine whether such a correlation also existed in fungi, especially since some of the phylogenetic and systematic relationships indicated here were already suggested by the analysis of the base composition of fungal RNA (15). Our calculations reveal that

such an overall correlation does not exist. However, for the 20 values of DNA with GC content below 50%, we found a positive linear correlation coefficient with a probability due to chance between 0.005 and 0.001. For the 15 values of GC higher than 50%, a negative linear correlation coefficient was found with a probability between 0.02 and 0.01.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-04431 from the National Institute of Allergy and Infectious Diseases.

We thank J. C. Alexopoulos, C. W. Hesseltine, and H. Phaff for kindly supplying some of the organisms. The expert advice of J. C. Alexopoulos in the selection of species and the technical assistance of Mrs. R. Morrill are gratefully acknowledged. Our gratitude also goes to M. Mandel for a generous supply of reference DNA.

LITERATURE CITED

1. BALDWIN, R. L., AND E. M. SHOOTER. 1963. The alkaline transition of BU-containing DNA and its bearing on the replication of DNA. J. Mol. Biol. **7**:511–526.
2. BELOZERSKY, A. N., AND A. S. SPIRIN. 1958. A correlation between the compositions of DNA and RNA. Nature **182**:111–113.
3. 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.
4. CHENG, T., AND N. SUEOKA. 1964. Polymer similar to polydeoxyadenylate-thymidylate in various tissues of a marine crab. Science **143**:1442–1443.
5. DOTY, P., B. B. MCGILL, AND S. A. RICE. 1958. The properties of sonic fragments of deoxyribose nucleic acid. Proc. Natl. Acad. Sci. U.S. **44**:432–438.
6. LODDER, J., 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.
7. MARMUR, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
8. MARMUR, J., AND P. DOTY. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
9. MARMUR, J., S. FALKOW, AND M. MANDEL. 1963. New approaches to bacterial taxonomy. Ann. Rev. Microbiol. **17**:329–372.
10. MESELSON, M., F. W. STAHL, AND J. VINOGRAD. 1957. Equilibrium sedimentation of macromolecules in density gradients. Proc. Natl. Acad. Sci. U.S. **43**:581–588.
11. MINAGAWA, T., B. WAGNER, AND B. STRAUSS. 1959. The nucleic acid content of *Neurospora crassa*. Arch. Biochem. Biophys. **80**:442–445.
12. ROST, K., AND H. VENNER. 1964. Untersuchungen an Nucleinsauren. X. Isolierung und Untersuchung von Deoxyribonucleinsaure aus Hefen. Z. Physiol. Chem. **339**:230–237.
13. 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.
14. SPIEGELMAN, S. 1961. The relation of informational RNA to DNA. Cold Spring Harbor Symp. Quant. Biol. **26**:75–90.
15. STORCK, R. 1965. Nucleotide composition of nucleic acids of fungi. I. Ribonucleic acids. J. Bacteriol. **90**:1260–1264.
16. URYSON, S. O., AND A. N. BELOZERSKY. 1960. Nucleotide composition of deoxyribonucleic and ribonucleic acids of certain fungi. Dokl. Akad. Nauk. SSSR **133**:708–710.
17. VANYUSHIN, B. F., A. N. BELOZERSKY, AND S. L. BOGDANOVA. 1960. A comparative study of the nucleotide composition of ribonucleic acid and deoxyribonucleic acids in some fungi and myxomycetes. Dokl. Akad. Nauk. SSSR **134**: 1222–1225.
18. VENNER, H. 1963. Isolierung und untersuchung von nucleinsauren aus basidiomyceten. Z. Physiol. Chem. **333**:5–19.
19. ZAMENHOF, S., AND E. CHARGAFF. 1950. Dissymmetry in nucleotide sequence of desoxypentose nucleic acids. J. Biol. Chem. **187**:1–14.