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Nucleotide Composition of Nucleic Acids of Fungi

I. Ribonucleic Acids

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ABSTRACT

STORCK, ROGER (The University of Texas, Austin). Nucleotide composition of nucleic acids from fungi. I. Ribonucleic acids. J. Bacteriol. **90**:1260–1264. 1965.—The nucleotide composition of the ribonucleic acids (RNA) present in extracts of 26 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 44.1 to 60.5 in a distribution composed of 8 species of zygomycetes, 10 of ascomycetes, 11 of deuteromycetes, and 8 of basidiomycetes. The GC-content range and average were, respectively, 44.1 to 49.3 and 46.4 for the zygomycetes, 47.4 to 54.4 and 50.2 for the ascomycetes, 48.2 to 54.5 and 51.6 for the deuteromycetes, and 50.4 to 60.5 and 52.4 for the basidiomycetes. The GC content averaged 45.6 and ranged from 44.1 to 46.3 for four *Mucor* species. In addition, GC contents significantly lower than 50 were also encountered in some species of Hemiascomycetidae, suggesting that AT type RNA is not uncommon in fungi. It was proposed that the base composition of fungal RNA might have a taxonomic and phylogenetic significance.

Information on the base composition of fungal ribonucleic acid (RNA) stems primarily from the work of Uryson and Belozersky (1960) and of Vanyushin, Belozersky, and Bogdanova (1960). These authors analyzed a total of 12 species distributed among myxomycetes, phycomycetes, ascomycetes, and deuteromycetes, and concluded that to a certain extent the nucleotide composition of total RNA (ribosomal RNA plus transfer RNA plus informational RNA) was species-specific. These authors found that the compositional diversity in RNA was smaller than in deoxyribonucleic acid (DNA) and that the guanine plus cytosine content (GC) of RNA, when expressed in moles per cent, was distributed between 50 and 55%. One species belonging to the genus *Absidia*, however, had RNA characterized by 44.8% GC. This value was the lowest ever reported and, together with 47.2% GC found by Elson and Chargaff (1955) for *Saccharomyces cerevisiae*, suggested that the base composition of total RNA from some fungi might be unique.

In the present work, 23 additional species belonging to four classes of eumycotina have been analyzed. The results clearly show that RNA preparations with GC content of less than 50% are common in fungi. In addition, our values, together with those found in the literature, suggest that the base composition of RNA might have a limited but significant systematic and phylogenetic value for this important group of microorganisms.

MATERIALS AND METHODS

Organisms. Organisms were selected in a manner which provided about equal representation from each class, and included yeast as well as filamentous forms. In each class, from two to four species belonging to the same genus were analyzed to determine the degree of similarity of their RNA composition.

Growth and harvest. All organisms were grown at 25 C in submerged cultures, under forced aeration, and in a medium eliciting a maximal rate of growth. In most cases, the inoculum consisted of a mycelial starter and, in some, of a homogenized spore suspension (Storck, 1963). The cultures in the growth phase were harvested by centrifugation or filtration. Pellets and mycelial mats were washed with 0.01 M tris(hydroxymethyl)aminomethane(Tris)-HCl buffer, *p*H 7.4, containing 0.01 μmole/ml of $MgCl_2$. Extracts were obtained by grinding with washed 50-mesh sand in chilled mortars, and were cleared of large debris by centrifugation at 15,000 × *g* for 15 min at 3 C. Although the pellets contained RNA, they were discarded because it was found with *Neurospora crassa* and *Aspergillus niger* that deoxycholate extracts containing all the RNA present in these pellets had the same base composition as the RNA in the supernatant fluid. A similar result was obtained with *Mucor rouxii*; the totality of the RNA was recovered after the treatment with ribonuclease of intact cells which had been precipitated with cold

trichloroacetic acid. Furthermore, as will be shown in the Results, our base ratio values were in agreement with those of others who analyzed the RNA after direct hydrolysis of the cells. It was therefore concluded that the supernatant fluids analyzed in the present work represent valid samples of the total RNA.

Determination of nucleotide composition. The procedures used for alkaline hydrolysis of RNA and chromatographic separation of nucleotides have been previously described in detail (Henney and Storck, 1963*a*). Dowex 1 X 8 (200 to 400 mesh) columns (height, 2 to 5 cm; diameter, 1.2 cm) in the formate form were used for amounts of RNA varying between 2.0 and 5.0 mg. The stepwise elution of the nucleotide was recorded automatically with a Gilford multiple sample absorbance recorder attached to a Beckman DU spectrophotometer. Three hydrolysates were analyzed simultaneously. Flow cells of 1-cm light path and a volume of 0.1 cm^3 were used. To prevent the formation of air bubbles which interfered with optical density measurements, ice-cold water was circulated through the jacket surrounding the cell chamber. The areas under the curves for each nucleotide in the elution diagram were measured with a planimeter. The volume of eluent and the time required for the elution of each nucleotide were determined to obtain, in each case, an average value for the flow rate. The amount of each nucleotide was found by multiplying the area under the curve, by the reciprocal of the molar extinction coefficients (Beaven, Holiday, and Johnson, 1955), and by the flow rate. The amount of each nucleotide was obtained independently from a direct analysis of the collected eluates. The values found with the automatic procedure for the simultaneous analysis of three hydrolysates were in close agreement with

TABLE 1. *Nucleotide composition of RNA from fungi**

Organism	No.†	Moles per 100 moles of identified nucleotides				
		C	A	U	G	G + C
Zygomycetes						
Absidia glauca	3	19.2 ± 0.1	26.1 ± 1.1	26.3 ± 0.4	28.4 ± 1.6	47.6
Mucor racemosus (1608)	6	19.2 ± 0.2	27.9 ± 0.1	27.5 ± 0.3	25.4 ± 0.2	44.6
M. rouxianus (4855)	6	20.4 ± 0.5	27.3 ± 0.6	26.4 ± 0.7	25.9 ± 0.5	46.3
M. rouxianus (8097)	6	19.5 ± 0.9	28.3 ± 0.6	27.6 ± 0.8	24.6 ± 1.5	44.1
M. rouxii (1894)	8	19.6 ± 0.2	27.1 ± 0.2	26.6 ± 0.2	26.7 ± 0.3	46.3
M. subtilissimus (1743)	8	19.6 ± 0.7	27.7 ± 0.5	26.0 ± 0.5	26.7 ± 0.4	46.3
M. subtilissimus (1909)	6	19.8 ± 0.3	26.9 ± 0.2	27.3 ± 0.3	26.0 ± 0.2	45.8
Phycomyces blakesleeanus	6	20.8 ± 1.5	27.8 ± 2.4	23.6 ± 1.7	27.8 ± 1.8	48.6
Zygorhynchus moelleri	2	20.0 ± 0.2	27.4 ± 0.0	26.9 ± 0.1	25.7 ± 0.1	45.7
Deuteromycetes						
Penicillium chrysogenum	3	21.5 ± 0.3	23.5 ± 0.6	24.5 ± 1.0	30.4 ± 0.3	51.9
P. notatum	3	22.8 ± 0.3	23.5 ± 0.3	23.8 ± 0.2	29.9 ± 0.1	52.7
Candida pulcherrima	3	23.1 ± 1.2	26.0 ± 0.4	22.8 ± 0.9	28.1 ± 0.9	51.2
Cryptococcus albidus	3	19.9 ± 0.6	22.1 ± 0.8	25.5 ± 1.0	32.4 ± 1.4	52.3
C. laurentii	3	21.6 ± 1.3	25.6 ± 0.4	23.3 ± 0.8	29.5 ± 0.6	51.1
Rhodotorula mucilaginosa	3	24.8 ± 1.6	30.9 ± 0.3	20.8 ± 0.5	23.4 ± 1.6	48.2
Torulopsis stellata	3	22.7 ± 0.6	27.5 ± 0.8	21.8 ± 1.4	27.6 ± 1.9	50.4
Ascomycetes						
Endomyces reesii	3	19.6 ± 0.1	26.8 ± 0.5	25.4 ± 0.7	28.2 ± 0.4	47.8
Debaryomyces kloeckeri	3	20.2 ± 0.7	27.2 ± 0.7	25.4 ± 0.4	27.2 ± 0.3	47.4
Lipomyces starkeyi	3	23.2 ± 1.3	23.0 ± 1.6	22.6 ± 0.5	31.3 ± 1.4	54.4
Pichia membranaefaciens	6	24.0 ± 1.9	24.8 ± 1.8	22.6 ± 1.2	28.6 ± 1.0	52.6
Saccharomyces cerevisiae	3	20.1 ± 0.6	26.6 ± 0.4	26.5 ± 0.6	26.7 ± 1.0	46.8
S. fragilis	2	21.9 ± 0.1	27.1 ± 0.8	25.4 ± 0.6	25.6 ± 0.1	47.4
Schizosaccharomyces octosporus	3	23.1 ± 0.6	24.0 ± 0.2	24.4 ± 0.6	28.4 ± 0.5	51.5
Basidiomycetes						
Sporobolomyces roseus	3	23.0 ± 0.4	25.0 ± 0.4	22.9 ± 0.4	29.1 ± 0.6	52.1
S. salmonicolor	6	22.8 ± 1.1	26.8 ± 2.1	21.6 ± 1.0	28.8 ± 1.9	51.7
Schizophyllum commune	4	23.4 ± 1.0	25.4 ± 1.0	22.5 ± 0.5	28.6 ± 1.1	52.0

* C, cytidylic acid; A, adenylic acid; U, uridylic acid; G, guanylic acid. The numbers in parentheses after the *Mucor* species refer to strains from the Northern Regional Research Laboratory, Peoria, Ill.

† Number of analyses.

TABLE 2. *Nucleotide composition of RNA from fungi as reported in the literature**

Organism	Moles per 100 moles of identified nucleotides					Reference†
	C	A	U	G	GC	
Zygomycetes						
Lichtheimia‡ species	19.5	28.8	26.4	25.3	44.8	1
Phycomyces blakesleeanus						
(−)	21.1	28.2	21.0	29.7	50.8	2
(+)	21.2	27.4	21.5	29.9	51.1	2
Deuteromycetes						
Aspergillus niger	23.9	24.2	21.9	30.0	53.9	3
	25.0	25.0	19.9	30.1	55.1	2
Botrytis cinerea	22.7	28.0	24.0	27.3	50.0	1
Penicillium stolonifer	23.6	24.9	24.7	27.0	50.6	4
Trichothecium roseum	23.7	25.8	20.8	29.7	51.4	1
Ascomycetes						
Neurospora crassa	23.7	24.7	24.2	27.4	51.0	5
	25.9	23.7	24.2	26.2	52.1	6
Helvella esculenta	22.3	25.7	23.6	28.4	50.7	1
Sclerotinia libertinia	21.9	28.0	21.8	28.3	50.2	2
Basidiomycetes						
Polyporus versicolor	25.9	20.0	19.4	34.6	60.5	7
Amanita muscaria	21.3	27.7	21.6	29.4	50.7	1
A. strobiliformis	22.2	24.1	24.9	28.8	51.0	7
Agaricus bisporus	21.6	24.2	22.4	28.8	50.4	2
Lycoperdon species	22.7	27.0	22.3	28.0	50.7	1

* C, cytidylic acid; A, adenylic acid; U, uridylic acid; G, guanylic acid.

† 1, Vaniushin et al. (1960); 2, Uryson and Belozersky (1960); 3, Moyer and Storck (1964); 4, Kleinschmidt and Manthey (1958); 5, Henney and Storck (1963); 6, Minagawa, Wagner, and Strauss (1959); 7, Venner (1963).

‡ *Absidia*.

those found in the literature and those obtained earlier in our laboratory (Henney and Storck, 1963*a*, *b*; Moyer and Storck, 1964).

RESULTS

The results of our determinations are shown in Table 1. For each nucleotide the value indicated represents the mean for two to eight analyses. The average deviation from this mean rarely exceeded ±2% and often was less than ±1%. In this respect, our results carry the same weight as those reported in the literature. The mean GC content is found in the last column. In most instances, the average deviation from this mean equals ±2% or less.

The nucleotide composition of RNA from fungi other than those investigated in the present work is presented in Table 2. For *Aspergillus niger* and *Neurospora crassa*, two sets of values have been included to permit a comparison of the results obtained in two laboratories. As seen for both organisms, the GC contents do not differ by more than 1.5%. The average GC for the (+) and (−) strains of *Phycomyces blakesleeanus* deviates from our corresponding value (Table 1) by 2.3%. Similarly, for *Saccharomyces cerevisiae*, our value of 46.8 is in agreement with the 47.2 reported by Elson and Chargaff (1955), with Crosbie, Smellie, and Davidson (1953), and with other values found in the literature: 47.7 (Midgley, 1962; Osawa, 1960), 48.4 (Fukuhara and Shortman, 1962), 51.2 (Kitazume and Ycas, 1963). This high value of 51.2 apparently results from the fact that potassium ions were not removed from the hydrolysates, a procedure which, according to these authors, causes a loss of guanylic acid by coprecipitation. If we exclude this last value from our comparison, the GC content of RNA from *S. cerevisiae* ranges from 46.8 to 48.4.

Since our results agree with those obtained in other laboratories, the GC-content values shown in Tables 1 and 2 were grouped. In Table 3, the average and range in GC content found for each taxonomic group are presented. The average value was used in the case of organisms for which several independent analyses were available. With the *Mucor* species, six strains belonging to four species were analyzed (Table 1). In total, 37 different species of fungi have been used for the calculations shown in Table 3.

TABLE 3. *Distribution of GC content of RNA among fungi*

Taxonomic group	No.*	Range†	Avg†
All classes	37	44.1–60.5 (16)	50.2
Zygomycetes	8	44.1–49.3 (5)	46.4
Ascomycetes	10	47.4–54.4 (7)	50.2
Basidiomycetes	8	50.4–60.5 (10)	52.4
Deuteromycetes	11	48.2–54.5 (6)	51.6
Mucor	4	44.1–46.3 (2)	45.6
Hemiascomycetidae	7	47.4–54.4 (7)	49.9
Euascomycetidae	3	50.2–51.6 (1)	50.8

* Number of species analyzed.

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

DISCUSSION

The GC content of fungal RNA ranges from 44 to 60%. The higher value was reported by Venner (1963) for one species of *Polyporus*. This unique composition reflects a content of 34.6% in guanylic acid. Further investigation of this genus and related genera is required to establish whether the compositional diversity of RNA from fungi is indeed greater than in the other groups of organisms. If *Polyporus* is omitted, the GC content varies from 44 to 55%. In absolute value, this variation is equal to the range for bacterial RNA (Belozersky and Spirin, 1960).

Vanyushin et al. (1960) found that the RNA from one *Absidia* (*Lichtheimia*) species contained 44.8% GC. The present work confirms this finding and further demonstrates that such values are not uncommon in fungi. Indeed, average GC contents of 46.9 ± 1.3 and 47.2 ± 0.5 were found, respectively, for the mucorales and for a group composed of one species of *Endomyces*, one of *Debaryomyces*, and two of *Saccharomyces*. A survey of the literature reveals that AT type RNA preparations are rarely encountered. No GC contents significantly lower than 50% were found for bacteria (Belozersky and Spirin, 1960) and for plants (Trim, Baker, and Leah, 1964) in studies of the same amplitude as the present one.

A small number of fungi have been analyzed. The significance of GC ranges and averages is therefore weak. If this is kept in mind, it is perhaps permissible to note that the GC distribution for the mucorales and the ascomycetes overlaps significantly. This overlapping results from the low GC content exhibited by the RNA from some Hemiascomycetidae. It is tempting to speculate on a possible link between the zygomycetes and the ascomycetes. This speculation is supported somewhat by the fact that the GC distribution of the mucorales and the basidiomycetes does not overlap.

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