Deoxyribonucleic Acid Base Compositions of Dermatophytes

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DNA was extracted and purified from 55 dermatophyte isolates representing 34 species of *Trichophyton*, *Microsporum* and *Epidermophyton*. The base compositions of the chromosomal DNA were determined by CsCl density gradient centrifugation and were found to be in the narrow range of 48.7 to 50.3 mol % G + C. A satellite DNA component assumed to be of mitochondrial origin was present in most strains, with a G + C content ranging from 14.7 to 30.8 mol % G + C. Heterogeneity in microscopic and colonial characteristics was not reflected in differences in the mean G + C content of the chromosomal DNAs. Strains varied in the G + C contents of satellite DNA, but these did not correlate with traditional species concepts.

INTRODUCTION

In distinguishing between species of dermatophytes, the diagnostic laboratory normally relies on morphological characteristics of the cultured micro-organism on agar and on a few simple biochemical tests (Rebell & Taplin, 1978). However, identification may be difficult as the micro-organisms tend to be variable in their appearance and some undergo the rapid and irreversible degenerative changes associated with pleomorphism. This may occur soon after isolation or on repeated subculturing. The colonies become white and fluffy and, like the microscopic morphology, are devoid of distinguishing features.

The purpose of this study was to determine if differences between species established by traditional criteria were reflected by differences in the molecular composition of their genetic material. It was considered that measurement of the mean DNA base composition of representative dermatophytes might permit more definitive and objective grouping of the species. DNA base compositions are an important and widely used characteristic in microbial classification, and the values are known for several medically important fungal genera, including species of *Aspergillus*, *Candida* and *Cryptococcus* (Normore, 1973). In this study, dermatophyte strains were selected to represent a range of morphological features and geographical origins. Attention was focussed on *Trichophyton rubrum* and *T. mentagrophytes* as these two species are commonly encountered in clinical situations and are often difficult to differentiate from one another.

METHODS

Organisms. The identities and sources of the test isolates are listed in Table 1. They originated from human infections in the United Kingdom, Republic of South Africa, Canada and New Zealand. Other cultures were obtained from the National Collection of Pathogenic Fungi (NCPF), maintained at the Mycological Reference Laboratory (MRL). Two non-dermatophyte species, *Scytalidium hyalinum* and *Hendersonula toruloidea* were included as they produce superficial infections clinically similar to those caused by derma-

tophytes and could be confused with them in specimen material (Gentles & Evans, 1970; Campbell & Mulder, 1977).

Growth conditions. The fungi were cultivated on malt agar slants [4 % (w/v) malt, 1.5 % (w/v) agar] containing chloramphenicol (5×10^{-5} g l⁻¹) to inhibit bacteria and cycloheximide (5×10^{-4} g l⁻¹) to inhibit non-dermatophytic fungi. The cultures were incubated at 26 °C for periods that varied from 2 d to several weeks, depending on the species, until there was sufficient growth to provide a seeding inoculum. This was used to inoculate 250 ml conical flasks containing 100 ml broth medium [1 % (w/v) peptone, 4 % (w/v) glucose, 0.1 % (w/v) Oxoid yeast extract, with chloramphenicol and cycloheximide] which were incubated at 26 °C on an orbital incubator at 100 rev. min⁻¹ for 4 to 14 d, depending on the strain. The primary cultures were then transferred to 2 l baffled conical flasks containing 500 ml broth medium; cycloheximide was excluded at this stage because of its slightly inhibitory effect on dermatophytes. The incubation period depended on the rate of growth. Mycelia, or arthrospores which were sometimes produced in abundance, were harvested by filtration after 5 to 12 d incubation. The yield ranged from approximately 20 to 50 g wet wt. Harvested growth was stored in plastic Petri dishes at -20 °C. Cultures were checked before harvesting to confirm that contaminants were absent and that the colonial and microscopic characters remained compatible with those of the species.

Crude extraction of DNA. About 30 g of the frozen harvested material was ground to a powder in a pre-chilled mortar, using liquid nitrogen (Mendonça-Hagler *et al.*, 1974). The triturated material was suspended in 100 ml buffer composed of 0.02 M-Tris and 0.01 M-EDTA, with the pH adjusted to 7.8 with 1 M-HCl.

DNA purification. The method used was a modification of the procedure described by Marmur (1961). Sodium dodecyl sulphate was added to the disrupted mycelial/arthrospore suspension to a final concentration of 1 % (w/v). This mixture was then agitated in a 500 ml conical flask at constant speed with a magnetic stirrer for 1 h at room temperature. Sodium perchlorate was then added to a final concentration of 1 M and the mixture was stirred for a further 1 h at room temperature. An equal volume of chloroform/isoamyl alcohol/90 % (v/v) phenol (24:1:25, by vol.) was added and the resulting emulsion was stirred for 2 h (Butterworth, 1976). The emulsion was then centrifuged at 18000 g at 4 °C for 10 min, and the upper, aqueous layer containing the DNA was removed. The impure DNA was precipitated overnight with 1.3 vol. chilled ethanol. The flocculent precipitate was collected by centrifuging at 10000 g at 4 °C for 10 min and redissolved in approximately 10 ml of 0.1 × SSC buffer (SSC buffer is 0.15 M-NaCl and 0.015 M-trisodium citrate, pH 7.0). The buffer concentration was adjusted to that of SSC and bovine pancreatic ribonuclease was added (final concentration 20 µg ml⁻¹) and incubated for 4 h at room temperature. Residual proteins were removed by treatment with chloroform/isoamyl alcohol (24:1, v/v) and the upper aqueous layer was then separated by centrifuging at 18000 g for 10 min at 4 °C. The DNA was selectively precipitated with chilled propan-2-ol in the presence of 0.3 M-sodium acetate and 0.001 M-EDTA. The DNA was redissolved in 0.1 × SSC buffer and stored at 4 °C over a few drops of chloroform. If necessary the DNA was deproteinized again with chloroform/isoamyl alcohol (24:1, v/v), and reprecipitated with 1.3 vol. chilled ethanol.

Estimation of base composition. Buoyant density of the DNA was determined by equilibrium centrifugation in a neutral CsCl density gradient (Meselson *et al.*, 1957). The DNA of *Micrococcus lysodeikticus* (Sigma) was used as a density marker; its buoyant density was taken to be 1.731 g ml⁻¹ (Schildkraut *et al.*, 1962). The mean DNA base composition (mol % G + C) was then calculated from the buoyant density by the equation of Schildkraut *et al.* (1962): $\rho = 0.098(\text{GC}) + 1.660$, where GC is the mole fraction of G + C, and ρ is the buoyant density (g ml⁻¹). The determination was repeated twice for each DNA sample and the mean value was used to calculate % G + C. DNA extractions from some strains were repeated to check the reproducibility between different batches of the same culture and the reliability of the extraction procedures. The base compositions of some strains were also estimated from melting temperature (T_m) measurements in 0.1 × SSC buffer according to the method of Marmur & Doty (1962) and calculated as described previously (Owen *et al.*, 1978).

RESULTS

For the majority of dermatophyte cultures the DNA preparation procedure yielded sufficient amounts of fibrous DNA (about 1 mg) for repeated G + C estimations. However, difficulties were sometimes experienced in that a flocculent DNA precipitate was obtained, which had to be collected by centrifugation. This was attributed to degradation of the DNA during trituration with liquid nitrogen.

The mean base compositions for the chromosomal DNA of strains are listed in Table 1. For all 55 strains, values were in the range 48.7 to 50.3 % G + C. Good reproducibility (± 0.4 % G + C) was found on repeated estimates for each strain; variations between different preparations were insignificant. Most of the strains tested had satellite DNA peaks

with lower G + C values, ranging from 14.7 to 30.8 % G + C (Table 2). The satellite DNA normally represented around 10 % of the total DNA extracted. However, the G + C content of the satellite DNAs from some strains varied on repeated DNA extractions and in repeated determinations on DNA from the same extraction. The commonest satellite peak was assumed to be mitochondrial in origin, whereas other peaks, when present, were possibly nuclear DNA or DNA contaminated with polysaccharide (Storck & Alexopoulos, 1970). The two clinically common species *Trichophyton mentagrophytes* and *T. rubrum* had almost identical mean G + C contents of 49.7 ± 1.0 % (8 strains) and 49.6 ± 0.5 % (8 strains), respectively. The other 14 species of *Trichophyton* had G + C values that differed by no more than 1 % from those of *T. mentagrophytes* or *T. rubrum*. Close similarities in G + C content were likewise found for the species of *Microsporum* (16 species had a mean G + C of 49.9 %).

Estimations of G + C contents from the melting temperature showed close agreement with those from the buoyant density technique and indicate that the chromosomal DNA probably did not have any unusual molecular features such as atypical bases or covalent modifications.

DISCUSSION

The dermatophytes examined in this study constituted a remarkably homogeneous group of micro-organisms with respect to their mean chromosomal G + C contents when compared with other fungi, in particular those of medical importance. Some genera, for example *Candida*, contain species with widely differing G + C contents (Storck & Alexopoulos, 1970): this variation is direct evidence of the confused taxonomic state of the genus.

The dermatophyte genera and species are defined and identified in the routine laboratory by traditional mycological criteria based on morphological characteristics and on a rather limited range of biochemical tests. In some cases, the differences used for classification purposes are rather trivial; for example, some species of *Trichophyton* are differentiated by minor variations in the structure of their microconidia. Other more recent approaches in which differences have been observed are based on nutritional requirements, mating compatibility with tester strains of sexually reproductive species and comparative analysis of antigens (Philpot, 1978). It must be assumed that such differences reflect only a small part of the information content of the genome because they do not appear to correlate with any obvious difference in the overall base compositions of the chromosomal DNA. Furthermore, although dermatophytes have become adapted to a wide range of environments, and have developed varying degrees of preference for human, animal and soil substrates, this has not been accompanied by any divergence in their G + C contents.

The principal objective of this study was to determine if differences between representative species of dermatophytes classified by orthodox mycological criteria were also manifested by differences in their DNA base composition. Our results showed that G + C contents of chromosomal and satellite DNA of the various dermatophyte species were essentially similar and therefore of no practical value in genus or species differentiation. The fact that the strains had almost identical chromosomal G + C contents indicated the likelihood of a high level of taxonomic similarity between the various genera and species. The G + C contents of the satellite DNAs varied between strains and consistent differences between species were not apparent so they had no application in classification. Studies with bacteria (Jones & Sneath, 1970), showed that the DNAs of organisms belonging to the same species should differ by no more than 3 to 4 % G + C, and those of organisms belonging to the same genus should differ by no more than 10 % G + C. Base compositions may therefore be used to exclude an organism from a taxon, but not as the sole criterion to include one. On this basis, our results for the dermatophytes do not contradict the present taxonomic arrangement, neither do they exclude any alternative arrangement in which dermatophytes could constitute a single genus of several species, or even a single species with several subspecies. We suggest, in view of the high overall degree of phenetic and genomic similarity exhibited

Table 1. *Dermatophyte species examined and mean DNA base composition*

Organism	Strain*	Buoyant density (ρ)	Base composition (mol % G + C)
<i>Trichophyton</i>			
<i>T. mentagrophytes</i>	MRL 9403	1.7089 $\pm$ 0.0001	49.9
	MRL 1619	1.7090 $\pm$ 0.0002	50.0
	MRL 9488	1.7092 $\pm$ 0.0001	50.2
	SA 4072	1.7086 $\pm$ 0.0001	49.2
	SA 4311	1.7088 $\pm$ 0.0000	49.8
	SA B1207	1.7077 $\pm$ 0.0000	48.7
<i>T. mentagrophytes</i> var. <i>interdigitale</i>	MRL	1.7086 $\pm$ 0.0002	49.6
<i>T. mentagrophytes</i> var. <i>granulare</i>	MRL	1.7091 $\pm$ 0.0001	50.1
Mean G + C content for <i>T. mentagrophytes</i> (8 strains) $\pm$ s.d.			49.7 $\pm$ 1.0
<i>T. rubrum</i>	MRL 6371	1.7090 $\pm$ 0.0001	50.0
	MRL 5034	1.7089 $\pm$ 0.0001	49.9
	MRL 6805	1.7085 $\pm$ 0.0000	49.5
	SJH Q3562	1.7088 $\pm$ 0.0002	49.8
	SJH Q3357	1.7086 $\pm$ 0.0000	49.6
	SJH R4079	1.7082 $\pm$ 0.0002	49.2
	SA C771	1.7081 $\pm$ 0.0000	49.1
	SA C1016	1.7083 $\pm$ 0.0001	49.3
Mean G + C content for <i>T. rubrum</i> (8 strains) $\pm$ s.d.			49.6 $\pm$ 0.5
<i>T. equinum</i>	MRL	1.7091 $\pm$ 0.0001	50.1
<i>T. tonsurans</i>	MRL 1924	1.7081 $\pm$ 0.0001	49.1
<i>T. erinacei</i>	MRL 3111	1.7092 $\pm$ 0.0001	50.2
<i>T. terrestre</i>	MRL 1811	1.7079 $\pm$ 0.0001	48.9
<i>T. gourvillii</i>	SJH EA767	1.7087 $\pm$ 0.0001	49.7
<i>T. soudanense</i>	SJH	1.7083 $\pm$ 0.0001	49.3
<i>T. fischeri</i>	NCPF 519	1.7091 $\pm$ 0.0001	50.1
<i>T. quinckeanum</i>	NCPF 342	1.7086 $\pm$ 0.0002	49.6
<i>T. gallinae</i>	NCPF 301	1.7077 $\pm$ 0.0001	48.7
<i>T. violaceum</i>	NCPF 401	1.7086 $\pm$ 0.0001	49.6
<i>T. megninii</i>	NCPF 303	1.7085 $\pm$ 0.0001	49.5
<i>T. ajelloi</i>	NCPF 369	1.7093 $\pm$ 0.0001	50.3
<i>Arthroderma benhamiae</i>	NCPF 411	1.7088 $\pm$ 0.0001	49.8
<i>Arthroderma simii</i>	NCPF 343	1.7088 $\pm$ 0.0004	49.8
<i>Microsporum</i>			
<i>M. canis</i>	MRL 1450	1.7087 $\pm$ 0.0001	49.7
	MRL	1.7081 $\pm$ 0.0001	49.1
<i>M. gypseum</i>	SJH R4173	1.7089 $\pm$ 0.0000	49.9
<i>M. audouinii</i>	SJH	1.7088 $\pm$ 0.0002	49.8
<i>M. fulvum</i>	NCPF 377	1.7086 $\pm$ 0.0001	49.6
<i>M. praecox</i>	NCPF 430	1.7084 $\pm$ 0.0002	49.4
<i>M. cookei</i>	NCPF 387	1.7087 $\pm$ 0.0003	49.7
<i>M. amazonicum</i>	NCPF 506	1.7088 $\pm$ 0.0001	49.8
<i>M. distortum</i>	NCPF 215	1.7090 $\pm$ 0.0001	50.0
<i>M. boullardii</i>	NCPF 474	1.7088 $\pm$ 0.0003	49.8
<i>M. persicolor</i>	NCPF 368	1.7083 $\pm$ 0.0001	49.3
<i>M. nanum</i>	NCPF 481	1.7082 $\pm$ 0.0001	49.2
<i>M. racemosum</i>	NCPF 483	1.7087 $\pm$ 0.0002	49.7
<i>M. ripariae</i>	NCPF 342	1.7084 $\pm$ 0.0000	49.4
<i>M. equinum</i>	NCPF 213	1.7087 $\pm$ 0.0001	49.7
<i>M. ferrugineum</i>	NCPF 334	1.7084 $\pm$ 0.0002	49.4
<i>Nannizzia gypsea</i>	NCPF 412	1.7092 $\pm$ 0.0003	50.2

Table 1. (Continued)

Organism	Strain*	Buoyant density (ρ)	Base composition (mol % G + C)
<i>Epidermophyton</i>			
<i>E. floccosum</i>	MRL	1.7088 $\pm$ 0.0001	49.8
	MRL 2011	1.7090 $\pm$ 0.0001	50.0
	MRL 2108	1.7089 $\pm$ 0.0000	49.9
	MRL 1613	1.7085 $\pm$ 0.0001	49.5
	SJH R1947	1.7092 $\pm$ 0.0002	50.2
	SJH ER6375	1.7087 $\pm$ 0.0002	49.2
	SJH ER6513	1.7092 $\pm$ 0.0002	50.2
Mean G + C content for <i>E. floccosum</i> (7 strains) $\pm$ s.d.			49.8 $\pm$ 0.4
<i>E. stockdaleae</i>	NCPF 472	1.7083 $\pm$ 0.0003	49.3
<i>Hendersonula toruloidea</i> (fast-growing strain)	SJH	1.7147 $\pm$ 0.0001	55.8
<i>Hendersonula toruloidea</i> (slow-growing strain)	SJH	1.7148 $\pm$ 0.0001	55.9
<i>Scytalidium hyalinum</i>	SJH	1.7151 $\pm$ 0.0002	56.2

* MRL, clinical isolate from the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine, London; SJH, clinical isolate from the Mycology Department, St John's Hospital for Diseases of the Skin, London; NCPF, stock strain from the National Collection of Pathogenic Fungi at the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine, London; SA, provided by Dr C. Young, Institute of Medical Research, University of Witwatersrand, Johannesburg, South Africa.

Table 2. Summary of the base compositions of chromosomal and satellite DNAs of dermatophyte genera

Genus	No. of strains	Base composition (mol % G + C)			
		Chromosomal DNA		Satellite DNA	
		Range	Mean $\pm$ s.d.	Range	Mean $\pm$ s.d.
<i>Trichophyton</i>	30 (16 species)	48.7–50.3	49.6 $\pm$ 0.44	21.4–30.1	24.6 $\pm$ 1.5
<i>Microsporum</i>	17 (16 species)	49.1–50.2	49.6 $\pm$ 0.41	14.7–30.8	25.5 $\pm$ 5.6
<i>Epidermophyton</i>	8 (2 species)	49.2–50.2	49.8 $\pm$ 0.32	16.0–28.8	28.0 $\pm$ 4.7

by the dermatophytes, that the distinctions made on existing mycological characteristics may not be fully justified. For a more precise understanding of the taxonomic relationships amongst the dermatophytes, the use of DNA base pairing procedures may be helpful in establishing the validity of the species as currently defined.

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