Nuclear and Satellite DNA Base Composition and the Taxonomy of *Saprolegnia* (Oomycetes)

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INTRODUCTION

The genus *Saprolegnia sensu stricto* (Dick, 1973) contains 12 'morphospecies' (see Scudder, 1974) separated from each other primarily on the basis of five key characters (Seymour, 1970): (i) presence or absence of antheridia; (ii) antheridial origin; (iii) presence or absence of pitting on the oogonial wall; (iv) presence of centric or subcentric oospores; and (v) oospore diameter.

The weight given to each of these characters varies for the different species and this has resulted in both broadly (e.g. *S. ferax*) and narrowly (e.g. *S. hypogyna*) defined species which may be separated from one another by differences in only one character. Furthermore, some important characters are culturally variable, some isolates apparently lose the ability to form oogonia in culture, and still other isolates have never been induced to form oogonia and therefore cannot be assigned to any species using the presently accepted criteria.

There are several problems associated with the accumulation of cytogenetic information for the genus so the extent to which morphological variations indicate genetic relationships is unknown (Dick, 1972; Dick & Win-Tin, 1973), but work by Storck & Alexopoulos (1970), Green & Dick (1972) and Clark-Walker & Gleason (1973) suggests that *Saprolegnia* species may have DNA base compositions that are sufficiently divergent to make this a useful character for separating isolates at the infrageneric level. In addition, the isolates examined by Green & Dick (1972) and Clark-Walker & Gleason (1973) contain a satellite DNA which might also be a useful taxonomic indicator.

METHODS

Isolates. We used 11 isolates of *Saprolegnia* (Table 1) assigned to the following species: *S. diclina* Humphrey, *S. hypogyna* (Pringsheim) de Bary, *S. parasitica* Coker, and *S. ferax* (Gruithuisen) Thuret, the type species. For the purposes of this communication we have not distinguished between *S. diclina* and *S. parasitica* and refer isolates assigned to these species to the *S. diclina*–*S. parasitica* complex. Two of the isolates (IBH and ATCC22284) had apparently lost the ability to produce oogonia in culture so their identities could not be confirmed: these have been retained in the species to which they were assigned by the investigators who originally isolated them.

To help rule out the possibility that the satellite DNA band was of viral origin, two isolates (GAN17 and GAN18) were examined using methods similar to those described by Volkoff, Walters & Dejardin (1972). Another species in the Saprolegniaceae, *Aphanomyces astaci* Schikora, may contain mycoplasma-like structures (Heath & Unestam, 1974), so this possibility was tested by isolating DNA from isolate IBH which has been examined

Table 1. Identity of the *Saprolegnia* isolates used for this study and the CsCl buoyant density and % GC of their DNA

Species	Source/no.*	Origin	Buoyant density (g cm ⁻³)†		% GC	
			Main band	Satellite	Main band	Satellite
<i>S. diclina</i> - <i>S. parasitica</i> complex	APCC204e	Marion Lake, British Columbia, Canada	1.718	1.708	59	49
<i>S. diclina</i> - <i>S. parasitica</i> complex	ATCC22284	Christiansburg, Virginia, U.S.A., from <i>Ictalurus punctatus</i>	1.717	1.707	58	48
<i>S. diclina</i> - <i>S. parasitica</i> complex	CBS282-38	England	1.718	1.708	59	49
<i>S. diclina</i> - <i>S. parasitica</i> complex	GAN17	Robertson Creek Hatchery (124° 59' W, 49° 20' N), British Columbia, Canada, from <i>Oncorhynchus nerka</i>	1.717	1.708	58	49
<i>Saprolegnia</i> sp.	GAN24	As GAN17	1.718	1.707	59	48
<i>Saprolegnia</i> sp.	GAN18	As GAN17	1.717	1.707	58	48
<i>S. ferax</i>	IBH	University of Leeds	1.718	1.707	59	48
<i>S. ferax</i>	APCC204a	Bedgebury, England	1.718	1.707	59	48
<i>S. ferax</i>	APCC204b	Lake District	1.718	1.708	59	49
<i>S. ferax</i>	ATCC26116 (RE47-15a)	University of California, Berkeley, U.S.A.	1.718	1.708	59	49
<i>S. hypogyna</i>	APCC206a	S.E. England	1.718	1.706	59	47

* Abbreviations: APCC, Aquatic Phycomycete Culture Collection, Department of Botany, University of Reading; all APCC cultures were sent by courtesy of Dr M. W. Dick; ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; GAN, G. A. Neish, Department of Botany, University of British Columbia, Canada; IBH, Dr I. B. Heath, Department of Biology, York University, Downsview, Ontario, Canada: this isolate is also deposited in APCC as no. 204g; RE, Dr Ralph Emerson, Department of Botany, University of California, Berkeley, U.S.A.

† The values given are the mean of two separate determinations. The range of buoyant densities for the main band was 1.716 to 1.718 g cm⁻³; for the satellite, 1.706 to 1.709 g cm⁻³. Individual results were accurate to ± 0.001 g cm⁻³.

extensively by electron microscopy and in which no mycoplasma-like structures have ever been detected (I. B. Heath, personal communication).

The cultures were tested for the presence of bacterial contamination by growing them in tryptone-yeast extract broth (Neish, 1975a).

DNA isolation and buoyant density determination. The method used for isolating the DNA was adapted from those of Green & Dick (1972) and Marmur (1961). The DNA buoyant density of one isolate (ATCC26116) has been determined previously (Clarke-Walker & Gleason, 1973) and was used to check our methods.

Mycelium, harvested by suction filtration, was washed with distilled water, frozen in liquid nitrogen, ground to a fine powder and suspended in diethyldithiocarbamate buffer (Green & Dick, 1972). To the suspension was added an equal volume of liquified phenol/suspension buffer (9:1, v/v), and sodium dodecyl sulphate to a final concentration of 0.02 g ml⁻¹. This suspension was stirred for 30 min, the phases were separated by centrifuging, and the aqueous phase was treated once with phenol and once with chloroform/isoamyl

alcohol to remove protein. Cold 95 % (v/v) ethanol was then added to the aqueous supernatant to precipitate the nucleic acids. The precipitate was dissolved in standard saline citrate (SSC) buffer (Green & Dick, 1972) and then incubated at 37 °C with T₁-ribonuclease, pancreatic ribonuclease and α -amylase (Green & Dick, 1972) followed by predigested pronase at 45 °C, and protein was again removed by treatment with chloroform/isoamyl alcohol. The DNA was precipitated with 95 % ethanol, dissolved in 0.1 $\times$ SSC and reprecipitated with isopropanol/acetate/EDTA (Marmur, 1961). This precipitate was collected by centrifuging, washed with ethanol, dissolved in SSC and stored frozen.

Ultraviolet absorption spectra (E_{max} , 257 nm; E_{260}/E_{280} , 1.80 to 1.96) and diphenylamine assays (Burton, 1968) were used to estimate the purity and concentration of the DNA preparations. DNA base compositions were determined from buoyant density measurements in caesium chloride (Schildkraut, Marmur & Doty, 1962).

RESULTS AND DISCUSSION

The main DNA component of all the isolates (Fig. 1; Table 1) had a buoyant density of 1.717 ± 0.001 g cm⁻³ (58 ± 1 % GC) which is within the range of experimental error. These results agree well with those obtained by Green & Dick (1972) for three isolates belonging to the *S. diclina*-*S. parasitica* complex, and by Clark-Walker & Gleason (1973) for ATCC26116, recently identified as *S. ferax* (Neish, 1975*b*). Our results are also in reasonable agreement with those obtained by Storck & Alexopoulos (1970) for *S. hypogyna* and *S. parasitica* (59, against their values of 55.5 and 60.5 % GC respectively). However, their report of 49.5 % GC for *S. ferax* is 9.5 % lower than our value for four different isolates of that species and, in fact, coincides with the position of our satellite component. This could not be due to experimental error in determination of the base composition, since they used the same buoyant density method. Unfortunately, this problem must remain unresolved since the isolate in question is no longer extant (E. S. Beneke, personal communication).

With this exception, the variation of base composition in the genus *Saprolegnia sensu stricto* appears to be at least within 5 % GC (55.5 to 60.5 % GC) and may be within 3 % GC (57 to 59 % GC). Clearly, analysis of DNA base composition cannot be used for separating species of *Saprolegnia* in accordance with classical morphological species concepts, but our data do suggest that *Saprolegnia* may be a relatively homogeneous taxon and that any isolate whose base composition is not between 55.5 and 60.5 % GC should be excluded from the genus. Further clarification of the infrageneric relationships of *Saprolegnia* species may have to be based on analysis of the DNA-DNA homologies between different isolates.

All isolates examined had a more or less well-defined satellite DNA with a buoyant density of 1.706 to 1.709 g cm⁻³ (47 to 50 % GC) (Fig. 1; Table 1). This satellite DNA was also found in three isolates of the *S. diclina*-*S. parasitica* complex by Green & Dick (1972), who showed that it was deoxyribonuclease sensitive and not due to contamination of cultures by bacteria or other fungi. The satellite was also reported by Clark-Walker & Gleason (1973) in *S. ferax* (ATCC26116). They showed that it was not mitochondrial DNA, which had a much lower buoyant density of 1.685 g cm⁻³. We did not detect any virus-like particles in the two isolates GANI7 and GANI8, both of which have distinct satellite bands. A mycoplasmal origin is unlikely because isolate IBH has a well-separated satellite peak, and no mycoplasma-like bodies have ever been detected in it. It is therefore most likely that this satellite DNA is of nuclear origin. Nuclear satellite DNA has been found in various eukaryotes (Walker, 1971; Ingle, Pearson & Sinclair, 1973) but has not been commonly reported for fungi (Storck, 1974).

Fig. 1. Microdensitometer tracings of the caesium chloride equilibrium gradients of the DNA of isolates of *Saprolegnia*. The numbers represent buoyant densities in g cm^{-3} . The peak on the right is reference DNA of *Micrococcus lysodeikticus* ($\rho = 1.731 \text{ g cm}^{-3}$). The lines in the figure approximate to the correct buoyant density; for precise values, see Table 1.

The amount of satellite DNA in the *Saprolegnia* isolates varied from about 20 % of the total DNA extracted (Fig. 1, GANI8) to about 5 % (Fig. 1, APCC204b). As yet we have no explanation for this phenomenon. It may be an artefact, represent real differences in the genome (Gibson & Hewitt, 1972), or be related to the physiological state of the organism at the time of harvest. For example, if the satellite DNA were coding for ribosomal RNA, the variation in amount could represent amplification of the rRNA genes (Ingle *et al.*, 1973). When the nature of the satellite component is better understood, it may prove to be of taxonomic value at the generic level.

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