

Short Communication

Characterization of the nuclear DNA of *Phialophora gregata* ff.sp. *adzukicola* and *sojae*

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Phialophora gregata nuclear (n) DNA was characterized by physical methods. The nDNA of f.sp. *adzukicola* was shown to be larger than that of f.sp. *sojae*, 2.9 and 2.1×10^{10} Da, respectively. The amounts of repetitive sequence and AT-rich region in the nDNA were also larger in f.sp. *adzukicola* than f.sp. *sojae*. These results indicate that the nuclear genome organization of the two formae speciales is differentiated.

Key Words—characterization of nuclear DNA; *Phialophora gregata* f.sp. *adzukicola*; *Phialophora gregata* f.sp. *sojae*.

The soil-borne fungus *Phialophora gregata* (Allington et Chamberlain) Gams causes brown stem rot on adzuki bean and soybean, and it was proposed that the fungus should be subdivided into two formae speciales based on their host specificity: f.sp. *adzukicola* for adzuki bean and f.sp. *sojae* for soybean (Kobayashi et al., 1991). This proposal is supported by the results of isozyme analysis (Yamamoto et al., 1990), GC content and DNA base sequence homology (Yamamoto et al., 1992). During the latter study, it was noticed that the nuclear genome organization of the two formae speciales of *P. gregata* was differentiated, and this is reported here.

Mycelia were prepared and disrupted as described previously (Yamamoto et al., 1990), and the nuclear (n) DNA was prepared as follows: whole-cell nucleic acids were extracted by the cetyltrimethyl ammonium bromide procedure (Murray and Thompson, 1980), from which RNA and mitochondrial DNA were removed by cesium chloride density gradient centrifugation in the presence of bisbenzimidazole (Garber and Yoder, 1983) and RNase A treatment.

DNA-DNA reassociation was examined as described by Kunita and Yokosawa (1982). The nDNA size was estimated from the $Cot_{1/2}$ value, where 50% of the DNA was reassociated in a sample (Britten and Kohne, 1968), using *Escherichia coli* DNA (Pharmacia) as a standard. Second-order rate plots of renaturing DNAs are shown in Fig. 1. The $Cot_{1/2}$ value of *P. gregata* nDNA was found to be approximately 10 times larger than that of *E. coli* DNA, indicating that the nuclear genome of *P. gregata* was 10 times more complex than *E. coli* DNA (2.8×10^9 Da). The genome sizes of *P. gregata* ff.sp. *adzukicola* and *sojae* were estimated to be 2.32–3.36 (average

2.88) and 1.85–2.26 (average 2.12×10^{10} Da, respectively (Table 1).

As shown in Fig. 1, *E. coli* DNA shows one line indicative of a single reaction rate, which means a repetitive DNA sequence is absent. However, *P. gregata* nDNA shows at least two lines with different slopes. Each line represents a distinctive fraction with a different degree of repetition, which is typical of higher organism nDNAs. The amount of repetitive sequence in the nDNA was calculated from second-order rate plot (Szecsi, 1981), and ff.sp. *adzukicola* and *sojae* nDNAs were estimated to contain 13.1–16.3 (average 14.2) and 9.9–12.9 (average 11.6)% of repetitive sequence, respectively (Table 1).

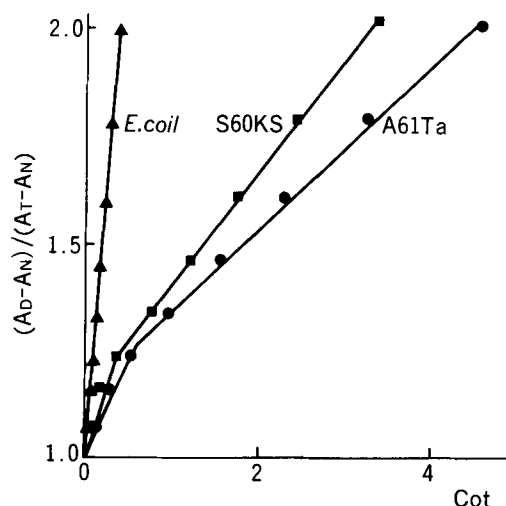


Fig. 1. Second-order rate plots of reassociation of nuclear DNAs from *Phialophora gregata* ff.sp. *adzukicola* (isolate A61Ta) and *sojae* (isolate S60KS) and of *Escherichia coli* DNA. A_D , A_N and A_T are absorbance of denatured, native DNA and renatured DNA at time T, respectively.

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Table 1. Genome sizes and amounts of repetitive sequence of nuclear DNAs from *Phialophora gregata* ff.sp. *adzukicola* and *sojæe*.

Isolate	Genome size ($\times 10^{10}$ Da)	Repetitive sequence (%)
f.sp. <i>adzukicola</i>		
A54-13	2.71	14.0
A54-24	2.84	13.1
A54W	3.18	16.3
A59To	3.36	13.9
A61Ta	2.84	14.1
A62B2-2	2.32	13.9
f.sp. <i>sojæe</i>		
S8001	2.18	9.9
S8302	1.85	12.6
S60KS	2.10	10.9
S62Ha-3	2.00	11.9
R-3	2.26	11.5
St-4	2.31	12.9

Thermal melting curves of the nDNA were obtained as described by Kuninaga and Yokosawa (1982). As shown in Fig. 2, *P. gregata* nDNA displays a biphasic curve, whereas that of *E. coli* DNA is uniphasic. The first derivative of the *P. gregata* nDNA melting curve means that the AT-rich region of the nDNA melts at a lower temperature and has a low GC content of ca. 30 mole%. Here, the amount of AT-rich region in the nDNA was evaluated as the proportion of the increase at 80°C to the final increase in the thermal melting curve, and those of *P. gregata* ff.sp. *adzukicola* and *sojæe* were 30–37 and 22–25%, respectively (Table 2).

It has been reported that the nuclear genome sizes of fungi fall in the range $0.6\text{--}3.0 \times 10^{10}$ Da and that fungal nDNA contains 5 to 20% of repetitive sequence (Szecei, 1981). In this study, *P. gregata* was found to fall within these ranges. The nuclear genome size varied widely depending on the isolate, but this should partially be attributed to errors in measurement. Therefore, the nDNA size

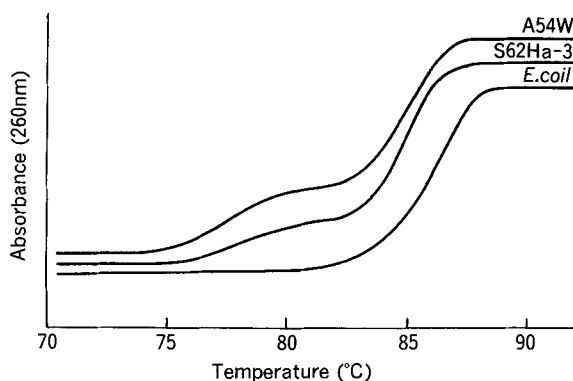


Fig. 2. Thermal melting curves of nuclear DNAs from *Phialophora gregata* ff.sp. *adzukicola* (isolate A54W) and *sojæe* (isolate S62Ha-3) and *Escherichia coli* DNA.

Table 2. Amounts of AT-rich region in nuclear DNAs from *Phialophora gregata* ff.sp. *adzukicola* and *sojæe*.

Isolate	Amount of AT-rich region (%) ^{a)}
f.sp. <i>adzukicola</i>	
A54-13	32.5
A54-24	30.3
A54W	32.5
A59To	36.5
A61Ta	35.5
A62B2-2	36.7
f.sp. <i>sojæe</i>	
S8001	21.8
S8302	22.5
S60KS	21.5
S62Ha-3	22.2
R-3	23.7
St-4	25.4

^{a)} The proportion of the increase at 80°C to the final increase in thermal melting curve.

of each isolate must be confirmed by other methods. However, the nuclear genome of f.sp. *adzukicola* appeared to be larger than that of f.sp. *sojæe*. The number of isozymes of lactate dehydrogenase and α -esterase was larger in f.sp. *adzukicola* than in f.sp. *sojæe* (Yamamoto et al., 1990), which may reflect the difference in the nuclear genome size. It was also shown that the amount of repetitive sequence and that of AT-rich region in the nDNA of f.sp. *adzukicola* were larger than those of f.sp. *sojæe*. The AT-rich and repetitive sequences in *P. gregata* nDNA could contain ribosomal (r) DNA, since it has been reported in other fungi that rDNA is tandemly repeated and has a low GC value (Garber and Yoder, 1983; Klassen et al., 1987). These results suggest that the nuclear genome organization differs between the two formae speciales.

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