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Short Communication

dsRNA viruses in *Nadsonia fulvescens*

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Double-stranded (ds) RNA viruses are now recognized as common in yeasts and have been reported in species of the genera *Cryptococcus*, *Cystofilobasidium*, *Hanseniaspora*, *Saccharomyces*, *Sporidiobolus*, *Trichosporon* and *Zygosaccharomyces* (Golubev et al., 2002, 2003; Kutaitè and Čitavičius, 1988; Pfeiffer et al., 1998; Schmitt and Breinig, 2002; Wickner, 1996). Typically these viruses are found associated with mycocinogeny (killer phenomenon), and two types of separately encapsidated dsRNA can be found in the cytoplasm of infected cells. The smaller one is responsible for mycocin production, while the other encodes the dsRNA-dependent RNA polymerase and the capsid protein for both viruses. In some cases (*Dipodascus*, *Xanthophyllomyces*), mycocinogenic activity was not found and the biological role of the dsRNA viruses remains unknown (Nosek et al., 1993; Pfeiffer et al., 1996). The phenotypic consequences of one type of dsRNA in *Candida curvata*, *Wickerhamia fluorescens* and *Yarrowia lypolitica* VLPs are also obscure (Matte et al., 1990; Pospisek et al., 1996; Treton et al., 1985).

This report describes the identification and initial characterization of dsRNA viruses in the apiculate pedogamic yeasts, *N. fulvescens* var. *fulvescens* and *N. fulvescens* var. *elongata*. The first variety is very rare, whereas the second one is a common inhabitant of spring sapwood fluxes of deciduous trees in temperate zones (Golubev et al., 1977).

Seven strains of two species, *N. commutata* and *N. fulvescens*, were examined for the presence of extrachromosomal genetic elements. Strains used in this study are listed in Table 1. Total nucleic acid was isolated from 3-day-old cultures (Pfeiffer et al., 1996). No extrachromosomal bands were found in *N. commutata* strains. One extrachromosomal band was visualized by agarose gel electrophoresis in two of the strains, *N. fulvescens* var. *elongata* VKM Y-2531 and *N. fulvescens* var. *fulvescens* VKM Y-2618 (Fig. 1). On the basis of their resistance to DNase (RNase free, SIGMA, St. Louis, MO, USA) and S1 nuclease (SIGMA) and their sensitivity to RNase (SIGMA), they were identified as dsRNA molecules (Fig. 2). Molecular weights, calculated from the marker *Hind*III-digested λ DNA (Marker 2, Fermentas AB, Vilnius, Lithuania) with the correction suggested by Livshits et al. (1990), are 4.95 kb in *N. fulvescens* var. *elongata* VKM Y-2531 and 6.02 kb in *N. fulvescens* var. *fulvescens* VKM Y-2618. RNase protection assay, carried out from the crude homogenate of the strains (Golubev et al., 2002), indicated the presence of protein capsid in both cases (Fig. 3). Therefore, samples were subjected to electron microscopic analysis. Isometric virus-like particles with diameters of approximately 28 nm in strain VKM Y-2531 and 30 nm in VKM Y-2618 were revealed (Fig. 4). The dsRNAs were co-purified with the VLPs in both cases, which means that these

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Table 1. Strains examined.

Species, variety	Strains	Source
Nadsonia commutata	VKM Y-1573T	From soil, East Falkland
Nadsonia commutata	VKM Y-2610	From soil, Carpatians, Ukraine
Nadsonia fulvescens var. *elongata*	VKM Y-268	From birch sap, Moscow region, Russia
Nadsonia fulvescens var. *elongata*	VKM Y-1653	From birch sap, Moscow region, Russia
Nadsonia fulvescens var. *elongata*	VKM Y-2531T	From birch sap, Smolensk region, Russia
Nadsonia fulvescens var. *fulvescens*	VKM Y-2532T	From oak sap, St.-Petersburg, Russia
Nadsonia fulvescens var. *fulvescens*	VKM Y-2618	From All-Union Institute of Agricultural Microbiology, St.-Petersburg, Russia

T, type strain; VKM, Russian Collection of Microorganisms, Pushchino.

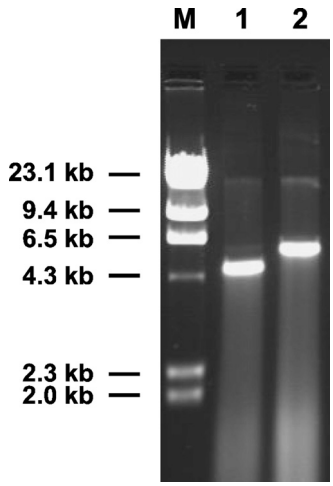

Fig. 1. Mini-lysate of *Nadsonia* strains.
M, *Hind*III-digested λ DNA (Fermentas); lane 1, strain VKM Y-2531; lane 2, strain VKM Y-2618.

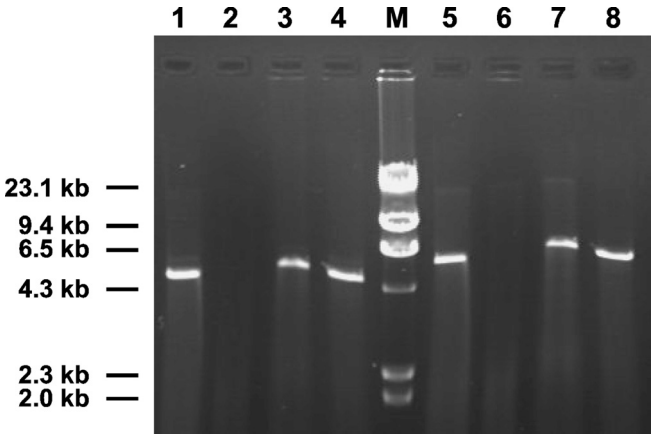

Fig. 2. Mini-lysate of the strains (lane 1, Y-2531; lane 5, Y-2618); after digested with RNase in TE buffer (lane 2, Y-2531; lane 6, Y-2618); S1 nuclease (lane 3, Y-2531; lane 7, Y-2618); DNase (lane 4, Y-2531; lane 8, Y-2618); M, *Hind*III-digested λ DNA.

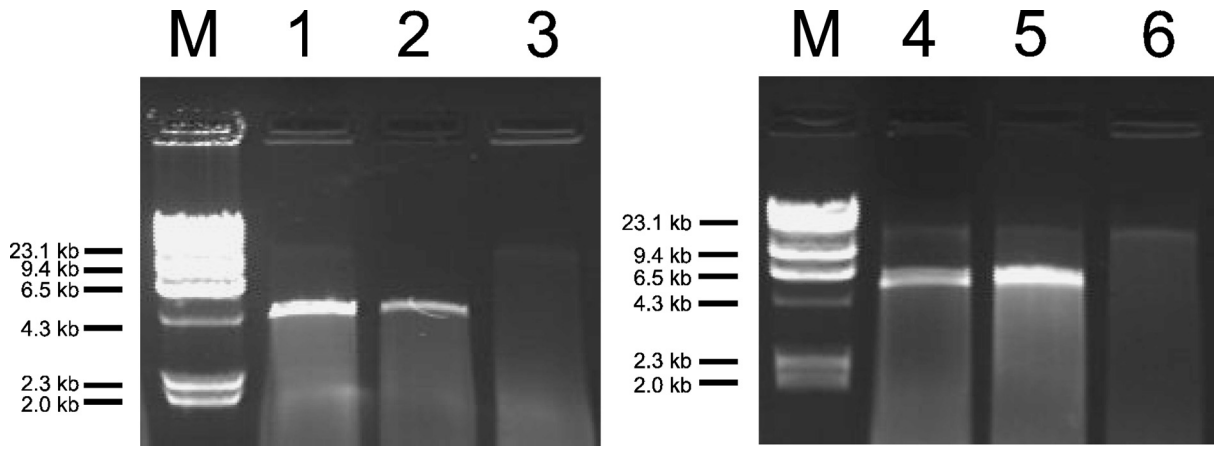

Fig. 3. RNase protection assay.
M, *Hind*III-digested λ DNA, crude homogenate of strains (lane 1, Y-2531; lane 4, Y-2618); crude homogenates digested with RNase before phenol-chloroform extraction (lane 2, Y-2531; lane 5, Y-2618); crude homogenates digested with RNase after phenol-chloroform extraction (lane 3, Y-2531; lane 6, Y-2618).

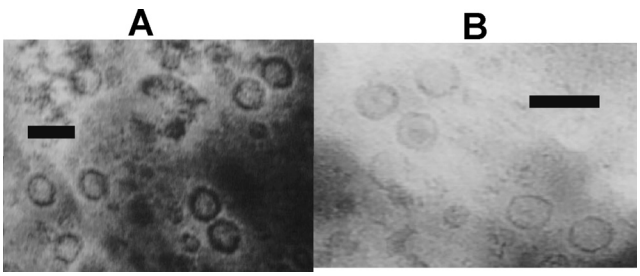

Fig. 4. Electron micrograph of VLPs from strains VKM Y-2531 (A) and VKM Y-2618 (B).

Samples were negatively stained by 2% uranyl-acetate and examined with a Zeiss OPTON EM902 electron microscope. Bar represents 50 nm.

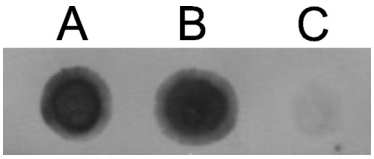

Fig. 5. Dot-blot hybridization.

A, strain VKM Y-2531; B, strain VKM Y-2618; C, *S. cerevisiae* T 158C.

molecules can form virus genomes and encode the proteins necessary for the maintenance of the viruses.

Relatedness of the VLPs of the *Nadsonia* strains and *S. cerevisiae* T 158C strain (exhibiting K1 phenotype) was investigated by dot-blot hybridization. dsRNA of the VKM Y-2531 strain was isolated from the gel by GenElute™ Agarose Spin Column (SIGMA) and labeled with digoxigenin molecules (DIG-Chem-Link Labeling and Detection Set, Roche Diagnostics GmbH, Mannheim, Germany). Strong homology was detected between the *Nadsonia* strains, while only a weak sign was observed in *S. cerevisiae* T 158C (Fig. 5), suggesting a close relationship between the VLPs of *N. fulvescens* varieties and a common evolutionary origin. Full-length sequencing could map the location of homologous regions, also interpreting the cause of the differences in molecular weight among the dsRNAs.

Twenty-four strains of the genus *Nadsonia* from the Russian Collection of Microorganisms were examined for mycocinogenic activity by cross-testing (glucose-peptone agar, pH 4.0 and 4.5, at 18°C, Golubev et al., 2002) within *N. commutata* and *N. fulvescens*. No activity has been detected in any strains, including the strains VKM Y-2531, Y-2618 and VKM Y-1653, VKM Y-268 where the presence of linear DNA plasmids was detected (Fejfár et al., 2003), thus the biological significance of *Nadsonia* dsRNA viruses as in the case of DNA plasmids remains obscure. *N. fulvescens* strains are rather variable in colony morphology and sugar fermentation (Golubev et al., 1989); thus the contribution of extrachromosomal genetic elements to phenotypic variability is open to speculation. It is interesting to note that dsRNA viruses are present in all dominant members (*Tr. pullulans*, *X. dendrorhous* and *N. fulvescens* var. *elongata*) of the yeast community in spring tree exudates (Golubev et al., 2002; Pfeiffer et al., 1996).

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