Isolation of dsRNA-associated VLPs from the strain
Cryptococcus hungaricus CBS 6569

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Abstract

Double-stranded RNAs (dsRNAs) with molecular masses 1.7 and 5.0 kbp, respectively, were isolated from the strain Cryptococcus hungaricus CBS 6569. The dsRNAs were copurified with icosahedric virus-like particles, 29 nm in diameter. This strain produced a protease-sensitive ‘toxin’ which inhibited the growth of strain C. hungaricus CBS 4214. The toxin had maximum activity at pH 3.7. The highest toxin amount was attained after a culture period of four days.

Keywords: Cryptococcus hungaricus; Double-stranded RNA; Virus-like particle; Mycocin

1. Introduction

Despite the increasing number of double-stranded RNA (dsRNA) viruses isolated from yeasts [1–5], still little is known about their role in the life of the host species. Their function is defined in two cases. In Saccharomyces cerevisiae [6] and Ustilago maydis [7] they confer the killer phenotype of the dsRNA-containing strains. In both cases the viruses code a toxin which destroys the cells of the sensitive strains of the same species. Many other yeast strains express killer phenotype [8], but up to now there is no direct evidence which shows that the toxin (mycocin) production is associated with the presence of extrachromosomal genetic elements.

This paper reports the presence of dsRNA associated virus-like particles (VLPs) in the strain Cryptococcus hungaricus CBS 6569 and demonstrates its mycocin production property. This species was isolated by Zsolt [9] as Dioszegia hungarica and was renamed and placed in the genus Cryptococcus by Fell and Phaff [10].

2. Materials and methods

2.1. Strains and culture conditions

Cryptococcus hungaricus CBS 4214, CBS 5124, CBS 6324, CBS 6569, CBS 6576 and CBS 6953 were employed in these experiments. Strains were
grown in 2×YPD medium (2% yeast extract, 2% peptone and 2% glucose) at 20°C with shaking at 200 rpm.

2.2. Purification and characterization of dsRNAs

dsRNAs were isolated from 4-day-old cells according to a previously published method [11]. Agarose gel electrophoresis was carried out as described [12]. The molecular masses of the dsRNA molecules were calculated by using the DNA size marker 4 (Fermentas). The molecular masses of the M and L dsRNA of the strain Saccharomyces cerevisiae T158C measured under the same conditions were 1.80 and 4.54 kbp, respectively.

The samples were digested with the following enzymes: ribonuclease A (50 μg ml⁻¹) in TE buffer; desoxyribonuclease (50 μg ml⁻¹) at 37°C for 30 min; and S1 nuclease (10 U ml⁻¹) at 37°C for 30 min in the recommended buffer [12].

2.3. Preparation of VLPs from crude homogenate

Four-day-old cells grown in 2×YPD medium were washed in ice-cold 0.1 M phosphate buffer (pH 7.4). They were then resuspended in the same buffer and were broken down by French-pressure (16000 Ψ). Unbroken cells and cell debris were then pelleted by centrifugation (20 min, 25000×g, 4°C). The supernatant (crude homogenate) was used for RNase protection assay and purification of VLPs.

The supernatant was extracted with phenol-chloroform and was then digested with RNase (50 μg ml⁻¹, for 30 min at 37°C). In the other case the RNase treatment was carried out under the same conditions before phenol-chloroform extraction.

VLPs isolated by the method of Oliver et al. [13] were negatively stained with 2% uranyl acetate and examined with a Zeiss OPTON EM902 electron microscope.

2.4. Testing of killer activity

The killer activities of Cryptococcus hungaricus strains were tested against each other. The procedures were carried out according to a previously described method [14].

3. Results and discussion

3.1. Isolation of dsRNAs

Six strains of C. hungaricus derived from the CBS collection were examined for the presence of dsRNAs. Agarose gel electrophoresis of the minily-sate of the strains showed two bands in addition to
the nuclear DNA in strain CBS 6569 (Fig. 1). The sensitivity of these bands to RNase and resistance to DNase (Fig. 2) and S1 nuclease, which degrades single-stranded nucleic acids specifically, confirmed that they are double-stranded RNA molecules. The estimated size of the smaller dsRNA was 1.7 kbp whereas the larger was 5.0 kbp.

3.2. Characterisation of VLPs

In most cases dsRNAs isolated from yeasts form the genome of virus-like particles. Therefore, RNase protection assay was carried out to demonstrate whether the dsRNAs of *C. hungaricus* are encapsi-

3.3. Mycocinogen activity of *C. hungaricus*

The presence of dsRNA viruses in *S. cerevisiae* is associated with the killer phenotype of the host strain. Therefore the mycocinogen activity of the *C. hungaricus* strains was tested. The strain *C. hungaricus* CBS 6569 carrying virus-like particles secreted a ‘toxin’ which inhibited the growth of strain *C. hungaricus* CBS 4214. The toxin activity was expressed in low pH range: 3.0–4.6, the maximum activity was measured at pH 3.7 (Fig. 5).

The killer activity of the strain was followed during the cultivation. It was noted that the toxin started to be produced on the second day and attained maximum activity on the fourth day, after which the activity decreased and stopped on the seventh day.

The killer factor’s resistance to proteases was dated or not. The result of this assay verified that these dsRNAs were resistant to RNase. This resistance could be destroyed by phenol-chloroform extraction (Fig. 3) suggesting that these dsRNA molecules are covered with protein molecules.

The electron microscopy of the dsRNA-containing fraction, separated by gradient centrifugation, revealed the presence of virus-like particles with icosahedric morphology 29 nm in diameter (Fig. 4).
tested. After incubating the sample with 10 μg ml⁻¹ proteinase K for 1 h at 37°C no inhibition effect could be observed, suggesting that the killer activity is caused by a proteinaceous compound.

All our attempts to eliminate dsRNA from *C. hungaricus* by known methods [15] failed up to now. Therefore, there is no direct evidence indicating that the toxin production is associated with the presence of dsRNAs.

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**References**


