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Virus-like particles from the yeast *Yarrowia lipolytica*

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Summary. Four out of the 24 strains of the yeast *Yarrowia lipolytica* we have checked for the presence of virus-like particles (VLPs) proved to contain encapsidated double-stranded RNA (dsRNA) molecules, 4.9 kb long. A major VLP polypeptide of MW 80,000 was observed in all 4 cases, and a second one of MW 77,000 in three cases. dsRNA from the VLPs harboring only the larger polypeptide showed little homology with the 3 others. We have found no homology between VLP dsRNAs and host DNA or dsRNAs from *Saccharomyces cerevisiae,* and no relationship between the presence of VLPs and a possible killer phenomenon in *Y. lipolytica.*

Key words: Virus-like particles – Double-stranded RNA – Yeast – *Yarrowia lipolytica*

Introduction

Non infectious virus-like particles have been observed in several yeast and fungi species (Lemke 1979). Most of them contain double-stranded RNA.

Among yeast genera, VLPs have been detected particularly in *Saccharomyces cerevisiae* (Adler and Mackenzie 1972) where they account for the widely investigated killer phenomenon (Herring and Bevan 1974; Wickner 1983a), but also in *Saccharomyces carlsbergensis* (Volkoff and Walters 1970), in *Candida tropicalis* (Nesterova et al. 1973, 1978; Kozlova 1973) in *Rhodotorula glutinis* (Nesterova et al. 1973), in *Candida albicans* (Mehta et al. 1982) and recently in *Yarrowia lipolytica* (Groves et al. 1983).

We have systematically examined twenty-four strains of the last mentioned species, and found VLPs in four of them. The present paper deals with their purification and some of their characteristics.

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Materials and methods

Organisms

Yarrowia lipolytica strains. CBS 599, CBS 2070, CBS 2072, CBS2073, CBS2074, CBS2075, CBS2078, CBS2787, CBS5570, CBS5589, CBS5699, CBS5919, CBS6012, CBS6114, CBS6124-1, CBS6124-2, CBS6125, CBS6303, CBS6317, CBS6614, CBS6660, CBS7033 and CBS7034 were obtained from the Central Bureau vor Schimmelculture, Delft, The Netherlands. IFP29 is an industrial strain.

Saccharomyces cerevisiae strains. A8209B, T158SK, T158DSK and T132B NK-1 were kindly provided by Dr. G. Fink, 1406 and 1384 by Dr. J. A. Bruenn. A8209B is a k1-killer strain, T158SK and T158DSK are two super k1-killer strains harboring an excess of M1-dsRNA, T132B NK-1 is a suppressive, non-killer strain. 1406 is a k1-killer strain and 1384 a k2-killer strain.

Growth conditions

Liquid medium. The cells were grown to stationary phase in YEPD medium (1% w/v yeast extract; 1% w/v peptone; 2% w/v dextrose) at 28 °C under slow agitation.

Solid medium. YEPD medium was supplemented with 1.4% to 2.5% w/v agar. For killer tests it was buffered from pH 4.5 to pH 6.5 with 0.05 M citrate-phosphate buffer.

Purification of VLPs from Y. lipolytica

The cells were harvested, washed and resuspended into their weight of TM buffer (0.01 M Tris-HCl, 0.001 M EDTA, 0.01 M $MgSO_4$, pH 8.0). All the subsequent operations were performed at 4 °C. The cells were broken with glass beads (0.45 mm diameter), 2 min at 2,000 rpm in a Braun homogenizer. The extract was cleared by centrifugation at 12,000 g for 30 min. The super-

natant was loaded onto 15 ml of a 45% w/v sucrose cushion (in TM buffer) and centrifuged overnight at 19,000 rpm in a SW28 Beckman rotor. The pellet was resuspended into TM buffer, and aggregates were dissociated with a Potter homogenizer. The suspension was brought to a density of 1.4 g/ml with CsCl and centrifuged at 35,000 rpm for about 48 h in a SW55Ti Beckman rotor. The bluish band of VLPs was collected by puncturing the side of the tube, diluted with TM buffer, adjusted to a density of 1.4 g/ml with CsCl and centrifuged as above. The band was collected and dialysed against TM buffer.

Purification of the VLPs from S. cerevisiae. It was performed as in Oliver et al. (1977).

Nucleic acid extraction

Extraction from VLPs. The VLP suspension was adjusted to 1% w/v SDS, then phenol extracted with a mixture of phenol-metacresol-8-hydroxyquinoline (Kirby 1968) until the interface was clear. The aqueous phase was treated with an equal volume of chloroform-isoamyl alcohol (24:1 v/v) and ethanol precipitated in the presence of 0.3 M sodium acetate or 0.3 M NaCl.

Extraction from cells. For rapid extraction (minilysates) of RNA, isolated colonies of yeast were grown at 28 °C for two to four days on solid medium, then scraped with a tooth-pick, and individually resuspended into 200 µl REB buffer (0.05 M Tris-HCl, 0.01 M EDTA, 0.1 M NaCl, 5% w/v SDS, pH 8.0). 100 µl phenol-metacresol mixture and 100 µl chloroform-isoamyl alcohol were added, then approximately 400 mg glass beads. Cells were broken by vigorous vortexing for 2 min, and centrifuged for 3–5 min in an Eppendorf centrifuge. The aqueous phase was transferred into a new tube, the organic phase was rinsed with 200 µl REB buffer and the second aqueous phase was added to the first one. After a second extraction with phenol-chloroform, traces of phenol were removed by two successive chloroform treatments. The aqueous phase was adjusted to 0.3 M NaCl and isopropanol precipitated.

Rapid extraction of DNA was as in Zamir et al. (1981), except that the cells were broken with glass beads.

Enzymatic tests

After ethanol precipitation, nucleic acid was pelleted, dried and redissolved into a small amount of adequate buffer, as indicated below.

DNase. Nucleic acid pellet wad dissolved into 0.01 M Tris-HCl buffer, pH 7.4, brought to 0.05 M $MgCl_2$ and subjected to DNase 1 from bovine pancreas (25 µg/ml) at 37 °C for 1 h.

RNase. Before use, pancreatic RNase was boiled for 10 min in 0.005 M Tris-HCl buffer, pH 8.0, in order to eliminate DNase contamination.

For strong treatment, nucleic acid was incubated at 37 °C for 1 h with RNase A from bovine pancreas (50 µg/ml) in 0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0.

Weak treatment was performed at room temperature for 10 min in either 2 x SSC, or 0.01 x SSC (1 x SSC : 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0) in the presence of 1 µg/ml RNase A.

S_1 nuclease. Treatment was performed with 5,000 U/ml S_1 nuclease from *Aspergillus oryzae*, at 37 °C for 1 h in 0.03 M sodium acetate, 0.001 M $ZnSO_4$, pH 4.6 (Vogt 1980).

Endonuclease from Neurospora crassa. Nucleic acid was incubated at 37 °C for 1 h in 0.1 M Tris-HCl, 0.001 M EDTA, pH 8.0, with 50 U/ml endonuclease.

Restriction endonuclease EcoR1. It was as in Maniatis (1982).

CF-11 cellulose chromatography

It was performed as in Franklin (1966).

Agarose gel electrophoresis of nucleic acid

Under non-denaturing conditions. Alcohol precipitated nucleic acid samples were pelleted, dried, resuspended into TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0) and run through 0.7% w/v agarose gel in TAE buffer (0.04 M Tris-acetic acid, 0.001 M EDTA, pH 7.7) after addition of 1/10 volume sterile loading buffer (bromophenol blue 0.25% w/v in 0.1 M EDTA, 1% w/v sarkosyl, 50% v/v glycerol, pH 8.1). After migration, the gel was stained with 1 µg/ml ethidium bromide in water and observed under UV light.

Under denaturing conditions. Samples were denatured by heating at 65 °C for 5 min in the presence of formaldehyde and formamide, and electrophoresed through 1.5% w/v agarose gel containing formaldehyde, as described in Maniatis (1982).

RNA transfer to Pall Biodyne A nylon membrane

After electrophoresis under denaturing conditions, samples were transferred to nylon membrane as in Thomas (1980).

DNA transfer to Pall Biodyne A nylon membrane

As in Maniatis (1982).

Hybridization with a radioactive probe

After RNA transfer, nylon membrane was dried under a lamp, baked at 80 °C for 1 h, and prehybridized at 42 °C for 4 h in the following mixture: 50% v/v desionized formamide, 5 x Denhardt's, 5 x SSPE, 0.3% w/v SDS, 250 µg/ml of sheared and heat denatured herring sperm DNA. (1 x Denhardt's: 0.02% w/v Ficoll of MW 400,000; 0.02% w/v polyvinylpyrrolidone of MW 260,000; 0.02% w/v bovine serum albumin. 1 x SSPE: 0.18 M NaCl, 0.001 M EDTA, 0,01 M sodium phosphate, pH 8.3). ^{32}P labeled probe was added, and hybridization was allowed overnight at 42 °C, then filters were washed as in Bostian et al. 1983. In some cases, two more 15 min washings at 65 °C were performed.

Labelling of RNA

VLP RNA was labeled with cytidine 3',5'-(5'-^{32}P) biphosphate (pCp) by T4-RNA ligase as in Welsh and Leibowitz (1982) Incubation was followed overnight at 4 °C. Enzyme was removed by phenol extraction and unincorporated radioactivity by Sephadex G50 chromatography (spun columns, as in Maniatis 1982). Before hybridization, the probe was denatured by boiling for 10 min, followed by quick chilling on ice.