Virus-Like Particles from Killer, Neutral, and Sensitive Strains of *Saccharomyces cerevisiae*

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Procedures were developed for purification of virus-like particles (VLPs) from killer, neutral, and sensitive strains of *Saccharomyces cerevisiae*. Morphologically similar spherical VLPs measuring 40 nm in diameter were extracted from all three phenotypes. The particles were partially purified by high-speed centrifugation through a layer of CsCl (1.26 g/cm³) and sucrose density gradient centrifugation. Gradient-purified preparations contained three centrifugal species that sedimented at approximately 43, 102, and 162S. The 43S component is considered to be an artifact. Preparations from killer strains contained three double-stranded RNA (ds-RNA) components with molecular weights of 1.19 × 10⁶, 1.29 × 10⁶, and 2.54 × 10⁶. VLPs from neutral and sensitive strains contained only the largest ds-RNA species. VLP preparations were subsequently separated into two major density components by CsCl equilibrium gradient centrifugation. The light component banding at 1.28 to 1.30 g/cm³ was void of nucleic acid, and the heavy component banding at 1.40 g/cm³ contained only the largest ds-RNA species.

In 1972, Adler and Mackenzie (Abstr. Annu. Meet. Am. Soc. Microbiol. G229, p. 8, 1972) reported the presence of isometric virus-like particles (VLPs), 40 nm in diameter, in cell-free extracts of *Saccharomyces cerevisiae*. Several other investigators subsequently observed similar particles in yeast cells (1, 5). Preliminary characterizations of these VLPs were reported by Buck et al. (5), Adler et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. V111, p. 219, 1974), and Herring and Bevan (8). These VLPs are of particular interest because of the extrachromosomal inheritance of the killer, neutral, and sensitive phenotypes of strains of *S. cerevisiae* (2, 12), suggesting an association between the inheritance of these phenotypes and the presence of VLPs. Killer strains produce a toxin that kills sensitive strains of yeast; neutral strains neither are inhibited nor produce killer toxin.

A double-stranded RNA (ds-RNA) species with a molecular weight of 2.5 × 10⁶ has been extracted from partially purified VLP preparations of both killer and sensitive strains; a lower-molecular-weight ds-RNA species, 1.4 × 10⁶, was detected only in VLP preparations from killer strains (8, 14). This suggested an association between the smaller ds-RNA species and toxin production. Similarly killer, neutral, and sensitive strains have also been reported in *Ustilago maydis*, in which the presence of mycovirus has been correlated with the cytoplasmically inherited factor for toxin resistance in that fungus (18).

The present investigation attempted to determine the optimum conditions for the extraction of VLP from the yeast cells and further characterization of these particles.

**MATERIALS AND METHODS**

**Cultures.** *S. cerevisiae* strains *1ad*, *2ad-leu*<sup>+</sup>, *3his*, *4ad*, *5trp-leu*<sup>+</sup>, *6his*, *7ad*, *8trp-leu*, *9ad*, and *11trp-leu* were obtained from Jacqueline Somers, McGill University, Montreal, Canada; *S. cerevisiae* strains Y502, Y3139, and Y960 were obtained from Morris Gordon, Division of Laboratories and Research, New York State Department of Health, Albany, N.Y.; *S. cerevisiae* 319A was obtained from Mildred Goldberg of the New York Hospital, Diagnostic Mycology Laboratory, New York City.

**Phenotype test.** The procedure of Wickner (15) was used to determine the killer, neutral, or sensitive characteristic of the strains used in this investigation.

**Growth media.** VLP extractions were made from cultures grown in Sabouraud broth (4% glucose, 1% peptone), GYP broth (2% glucose, 0.5% yeast extract, and 1% peptone [Difco]) (13), complete medium (CM) broth (4% glucose, 0.5% yeast extract, 0.3% peptone, and the following vitamins: 0.5 g of hydrolyzed casein, 5 mg of b- biotin, 10 mg of 1-
electron grids were gradient having solution phosphotungstate, at centrifuged at 212S, 152S, 115S, and cesium chloride. Some preparations floated at 4°C and 133,000 g. The VLPs were suspended in fractions through a 74,000 x g rotor. In experiments, before high-speed centrifugation, the pellets were resuspended, subjected to low-speed centrifugation, and again pelletted through a CsCl underlayer. The pellets were resuspended in buffer and centrifuged by low-speed centrifugation, and the supernatant solution was centrifuged at 4°C for 3 h at 105,000 x g.

Gradient centrifugation. VLP preparations were layered onto 10 to 40% sucrose density gradients (16) and centrifuged at 190,000 x g for 1.5 h at 4°C in a Beckman SW41 rotor. In some experiments, before sucrose density gradient centrifugation, the preparations were incubated for 1 to 2 h at room temperature with 5 μg of DNase per ml (Schwarz/Mann, Orangeburg, N.J., RNase free) in 0.01 M Tris-0.01 M KCl-0.0065 M MgCl2-6H2O buffer, pH 7.5.

Sedimentation velocities were estimated by log-linear sucrose density gradient analysis according to the procedure of Brakke and Van Pelt (4). The standards used were as follows: *Aspergillus flavus* (NRRL A-12268) VLPs, 49S (19); southern bean mosaic virus, 115S (11); and *Helminthosporium maydis* VLPs, 152S, 212S, and 283S (Bozarth and Wood, unpublished data).

Sucrose density fractions containing VLPs were concentrated and layered onto a cesium chloride solution having an initial density of 1.35 g/cm3 and centrifuged at 133,000 x g for 20 h at 4°C. Densities of the fractions were determined by refractometry (3).

Sucrose and cesium chloride density gradients were fractionated with an ISCO (ISCO, Lincoln, Neb.) density gradient fractionator and analyzer.

Electron microscopy. Carbon-coated Formvar grids were floated on drops of gradient fractions, rinsed with water, negatively stained with 2% sodium phosphotungstate, and examined in a Zeiss EM9-2S electron microscope.

Polyacrylamide gel electrophoresis. VLP gradients were incubated with 10% sodium laurel sulfate and 10% sucrose for 15 min at 60°C (10). They were then subjected to electrophoresis in 2.4% polyacrylamide gels (9) for 4.5 h at 6 mA/gel and scanned at 260 nm in a Gilford (Gilford Instruments, Oberlin, Ohio) model 2400-S spectrophotometer with a linear transport accessory. Double-stranded nucleic acid was detected by staining gels with 0.01% aqueous toluidine blue for 12 h and destaining with water (8).

Orcinol and diphenylamine tests. Sucrose density gradient VLP fractions were subjected to the orcinol and diphenylamine tests (7).

RESULTS

Growth conditions. During differential centrifugation, preparations from yeast cells grown in different media contained varying amounts of host materials that pelleted and resuspended along with the VLPs. VLP preparations from cells grown in GYP broth contained a large amount of this host material, whereas yeast grown in Saprobrus broth contained less. Growth of the cells in CM and modified CM broth resulted in the lowest relative quantity of contaminating host materials.

Purification. Precipitation of VLPs with polyethylene glycol (6,000 to 7,000 molecular weight) was obtained after one low-speed centrifugation. This procedure eliminated a significant amount of the contaminant material; however, such preparations were not suitable for further studies. Sufficient purification of the VLPs could be obtained by centrifuging the extracts through an underlayer of cesium chloride (1.26 g/cm3). Subsequent sucrose density gradient centrifugation eliminated most of the non-VLP materials, although cesium chloride equilibrium density gradient centrifugation was required to obtain highly purified VLP fractions.

VLPs could be extracted from the yeast cells by using either TKM buffer, pH 7.0, 7.5, 8.0, and 8.5, or sodium phosphate-NaCl buffer at pH 7.0 to 8.5. However, the particles were most stable in the 0.03 M sodium phosphate-0.15 M NaCl buffer at pH 7.5.

Screening. Each of the 14 yeast strains was assayed to determine its phenotype with respect to the killer, neutral, and sensitive characteristics. These strains were also assayed for presence of VLPs by electron microscopic examination of fractions from sucrose density gradients. VLPs were detected in all killer and neutral strains but not in all sensitive strains (Table 1). The relative yield of VLPs, as determined by sucrose gradient analysis, varied greatly within each phenotypic group. All particles were uni-
formly 40 nm in diameter when stained with 2% phosphotungstic acid (Fig. 1).

**VLP characterization.** Log-linear sucrose density gradient centrifugation indicated that all VLP preparations contained particles that sedimented at approximately 43S, 102S, and 162S (Fig. 2A). When sucrose gradient-purified VLP preparations from killer (8trp-leu-), neutral (6his-), and sensitive (2ad-leu2-) strains were centrifuged to equilibrium in cesium chloride, the 162S component banded predominantly at 1.40 g/cm³. The 43S and 102S particles banded as a group of peaks in the region of 1.28 to 1.30 g/cm³ (Fig. 2B). If the latter density fraction was subsequently centrifuged on sucrose density gradient-purified VLP-yielding strain examined. All cesium chloride peak fractions contained only VLPs when examined by electron microscopy.

**Nucleic acid characterization.** Polyacrylamide gel electrophoresis of the nucleic acids extracted from pooled VLP sucrose gradient fractions of the killer strain, 8trp-leu-, demonstrated the presence of three nucleic acid components. Positive orcinol tests and pink reactions upon staining with toluidine blue indicated that these components were ds-RNA. A small DNA peak was also observed when VLP preparations were not treated with DNase before nucleic acid extraction. Using *Penicillium chrysogenum* virus ds-RNA as molecular weight markers (17), the three ds-RNA components were found to have molecular weights of 2.54 × 10⁶, 1.29 × 10⁶, and 1.19 × 10⁶ (Fig. 3A). VLP preparations from neutral (6his-) and sensitive (2ad-leu2-) strains contained only the 2.54 × 10⁶-dalton species (Fig. 3B).

The 1.28- to 1.30-g/cm³ density component had an UV absorbance ratio of 0.95 at 260/280 nm, indicating a lack of nucleic acid. The 1.40-g/cm³ component had a 260/280 nm ratio of 1.60 and was found to contain only the ds-RNA with a molecular weight of 2.54 × 10⁶ (Fig. 3C).

**DISCUSSION**

Although the first report of VLPs in *S.

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**Table 1. Detection of virus-like particles in strains of Saccharomyces cerevisiae**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Virus-like particles</th>
<th>Detected</th>
<th>Relative concnᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2ad-leu₂</td>
<td>Sensitive</td>
<td>+</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>iad-</td>
<td>Sensitive</td>
<td>+</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>6his-</td>
<td>Sensitive</td>
<td>−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4ad-</td>
<td>Sensitive</td>
<td>+</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>11trp-leu-</td>
<td>Sensitive</td>
<td>+</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Y502</td>
<td>Sensitive</td>
<td>+</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Y3139</td>
<td>Sensitive</td>
<td>−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y960</td>
<td>Sensitive</td>
<td>+</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>8trp-leu₁</td>
<td>Neutral</td>
<td>+</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>6his-</td>
<td>Neutral</td>
<td>+</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>7ad-</td>
<td>Killer</td>
<td>+</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>8trp-leu-</td>
<td>Killer</td>
<td>+</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>9ad-</td>
<td>Killer</td>
<td>+</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>319A</td>
<td>Killer</td>
<td>+</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Relative concentration of VLPs per gram (wet weight) of cells estimated from sucrose density gradient absorption peaks (absorbency at 254 nm). Values were compared with that of strain 2ad-leu₂, the maximum VLP-yielding strain examined.

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**Fig. 1.** Electron micrograph of VLPs (1.28 to 1.30 g/cm³) extracted from *S. cerevisiae* and stained with 2% phosphotungstic acid.

**Fig. 2.** (A) Sucrose density gradient profile of VLPs extracted from a killer strain (8trp-leu-); (B) cesium chloride density gradient profile of pooled 43, 102, and 162S VLP-containing fractions from sucrose gradient.
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cerevisiae occurred over 2 years ago, purification of these VLPs has been difficult. This was primarily due to the occurrence of host contaminants that were not eliminated during centrifugal fractionation procedures. The parameters affecting the relative amount of these contaminants seemed to include the strain of the fungus, growth medium and conditions, and buffer used for extraction. It was observed in the present study that growth of the cells in CM, extraction of the VLPs with 0.03 M sodium phosphate-0.15 M NaCl buffer at pH 7.5, and high-speed centrifugation of the VLPs through an underlayer of CsCl (1.26 g/cm\(^3\)) improved the purity of VLP preparations from S. cerevisiae.

In the present study 12 of the 14 strains tested contained varying amounts of morphologically identical particles (Table 1) which in all 12 strains exhibited three components sedimenting at 43S, 102S, and 162S. These results and others (5, 8) indicated the widespread occurrence of VLPs in S. cerevisiae and suggest that common VLPs may be present in all of these isolates. The two fastest-sedimenting components were also observed by Buck et al. (5) and Herring and Bevan (8).

The nature of the 43S component that had a density of 1.28 to 1.30 g/cm\(^3\) is unclear. A 40-nm empty capsid of this density would not be expected to band at 43S without an association with low-density host material. The disappearance of this component after an intermediate purification step in cesium chloride gradients suggests such an association.

The 162S component that banded at 1.40 g/cm\(^3\) in cesium chloride contained ds-RNA with a molecular weight of 2.54 x 10\(^4\). The 102S component that banded at 1.28 to 1.30 g/cm\(^3\) contained no nucleic acids.

The use of CsCl equilibrium gradients for obtaining highly purified VLP fractions is questionable. Under these conditions there is an apparent breakdown of a large proportion of the 162S component (Fig. 2). The CsCl underlay procedure, however, does eliminate most of the host material without significant VLP degradation. This is indicated by the fact that all VLP preparations for a single strain exhibited a constant ratio of the three centrifugal components.

VLPs from killer, neutral, and sensitive strains of S. cerevisiae contained 2.54 x 10\(^4\)-dalton ds-RNA species. Partially purified preparations from a killer strain (8trp-leu-) contained two additional lower-molecular-weight ds-RNA species: 1.19 x 10\(^4\) and 1.29 x 10\(^4\). When these partially purified VLP preparations from the killer strain were pretreated with RNase in a low-molarity buffer before nucleic acid extraction, the smaller ds-RNA's were not recovered (unpublished data). These lower-molecular-weight ds-RNA's were also not present in nucleic acid extracts of VLP preparations.
that had been subjected to cesium chloride centrifugation (Fig. 3C). These results suggest that the smaller ds-RNA’s may be encapsulated within the VLPs, as indicated by Herring and Bevan (8), but particles containing these RNA species are preferentially degraded in cesium chloride equilibrium gradient centrifugation; alternatively, they may be unencapsulated nucleic acid, similar to the contaminant DNA detected in VLP preparations (Fig. 3).

Other investigators have observed a low-molecular-weight species of ds-RNA, $1.4 \times 10^4$, in either partially purified VLP preparations from killer strains (8) or in total RNA extracts of killer strains (1, 14). A correlation has been established between the presence of this ds-RNA species and the production of killer toxin (1, 8, 14). It should be noted, however, that killer strains have not been reported which contain only the highest-molecular-weight species of ds-RNA; therefore, it is possible that the presence of a VLP with the largest ds-RNA component may be necessary for maintenance of the smaller ds-RNA species.

ACKNOWLEDGMENTS

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LITERATURE CITED