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ISOLATION AND IDENTIFICATION OF A TEMPERATE PHAGE INDUCED FROM *Candida albicans*

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Abstract- A temperate phage was induced from *Candida albicans* ATCC 10231 using mitomycin-c at concentration of 1µg/ml. The temperate phage was designated *Candida albicans* induced phage (CAIP1). *Candida albicans* induced temperate phage (CAIP1) formed circular turbid single plaques of 2mm in diameter.

The susceptibility of twenty seven *C. albicans* and two *S. cerevisiae* isolates to the induced phage was tested. The induced phage was found to be infectious only to *Candida albicans* ATCC 10231 among the tested isolates.

Electron micrograph of negatively stained phage particles showed hexagonal icosahedral virus particles of 78 nm in diameter and may be classified under to family *Pseudoviridae, Genus Hemivirus.* This phage **(**CAIP1**)**, has been entered into databases at Phages DB. It can be viewed at the link: http://phagesdb.org/phages/CAIP1/

Ultrathin sections of infected *C. albicans* cells and healthy ones were examined by electron microscopy. Marked changes in mitochondria and nuclear membrane were observed in the infected cells as compared to the healthy ones. Moreover, in the late phase of infection, cytoplasmic coagulation, lysis of the cell wall and release of the induced phage particles were also observed.

Keywords- *Candida albicans*, Temperate phage and Transmission electron microscope

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Introduction

Candida albicans is an opportunistic human pathogenic fungus causing diseases of gastrointestinal and genitourinary tracts [1,2]. Also, *C. albicans* causes a various forms of candidiasis [2]. Infection with *C. albicans* which is associated with immune depressed patients and delayed treatment of systemic infections can lead to increased percentage of mortality [3].

The majority of mycoviruses are isometric particles with ds-RNA genomes [4], while approximately 30% of mycoviruses particles containing positive ss-RNA [5]. Over 90% of mycoviruses species covering ten viral families but 20% were unassigned to a genus or sometimes not even to a family [6]. Isometric forms predominant mycoviral morphologies were compared with other forms such as rigid rods, flexuous rods, enveloped bacilliform and herpes type particles [7]. The lack of genomic information often hampers a conclusive assignment to already established groups of viruses, it is impossible to erect new families and genera [5].

Mycoviruses families: *Chrysoviridae, Hypoviridae*, *Partitiviridae*, *Totiviridae, Reoviridae*, *Pseudoviridae* and *Narnaviridae* are dominating the icosahedral capsid structure [8-10].

The phage display technology based on identification of short peptide sequences that can distinguish *C. albicans* from other closely related species [11].

The hybrid phage displaying antigen epitopes is better, in the diagnosis of systemic *C. albicans* infection in rabbits and cancer patients, than the recombinant protein for its easy preparation and less expensive [12].

The present investigation aims to induce, characterize and identify temperate phages from lysogenic *C. albicans* isolates. In addition the histopathological effects and alteration of cell structure due to induction of the temperate phages were also studied.

Material & Methods

Source of Yeast Isolates

Twenty seven isolates of *Candida albicans* and two isolates of *Saccharomyces cerevisiae* were used in this study. *C. albicans* ATCC10231 was provided by Botany Department, Faculty of Science, El-Azhar University., and the other twenty six *C. albicans* isolates were provided by Botany and Microbiology Department., Faculty of Science, Helwan University. While the two *S. cerevisiae* isolates were obtained from Microbiology department, Faculty of Agriculture, Ain Shams University.

Yeast Cultivation

C. albicans isolates were grown on potato dextrose agar medium [13], while *S. cerevisiae* isolates were grown on malt extract agar medium at 30°C for 48 hrs. The isolates were maintained on the previous specific agar media slopes, stored at 4°C and sub-cultured at monthly [14].

Induction and Detection of Temperate Phage

Mitomycin-C at concentration of 1 mg/ml was used for induction of temperate phages from *C. albicans* and *S. cerevisiae* isolates as described by Franche [15].

Qualitative and Quantitative Techniques

The qualitative and quantitative tests for the prepared suspension of the induced phages were performed using spot test and plaque assay techniques, respectively, as described by Franche [15]. The single plaque isolation technique was used to purify the phage isolates.

Preparation of High Titer Yeast-phage Suspension

Liquid enrichment technique was used to prepare high titer phage lysate, according to Franche [15].

Host Range of the Induced Phage

Double layer agar plates were prepared. Each of the twenty seven *C. albicans* and two *S. cerevisiae* isolates was used as indicator hosts in individual plates. The surface of every plate was spotted with drops of the prepared high titer phage suspension. After incubation at 30°C for 48-72 h. the plates were examined for clearance at the sites where the drops were applied.

Electron Microscopy of the Induced Phage

Three ml of high titer phage suspension were purified by filtration through a 0.45mm disposable bacterial filter. One drop of the filtrated (0.45 mm filter) induced phage preparation was placed on a carbon coated copper grid for 2 min. The excess liquid was then removed with a filter paper wick. Grids were negatively stained with 2% (w/v) phosphotungestic acid (PTA) at pH 6.8 and examined with the transmission electron microscope (a Philips CM 400t, electron microscopy unit, El-Azhar Univ.).

Cytopathic Effects of the Induce Temperate Yeast-Phage on Yeast Vegetative Cells

Suspensions of healthy and phage infected cells of *C. albicans* ATCC10231 were centrifuged at 6000 rpm for 15 min at 4ºC. The pellets of the lysed and healthy cells were fixed for 4 h., in 0.08m CaColylate buffer (pH 7.4). Samples were post fixed in 1% osmium tetroxide for 3 h. Samples were dehydrated in ascending concentrations of alcohol series (50-90%) sequentially. Ultrathin sections were prepared using a microtome as described by Lo, et. al. [16], and Poranen & Bamford [17]. The sections were examined in Joel-Jem 1010, transmission electron microscopy (El-Azhar Univ. Unit).

Results

Induction of a Temperate Phage from Yeast Isolates

Twenty seven isolates of *Candida albicans* and two isolates of *Saccharomyces cerevisiae* were treated with mitomycin-C at concentration of 1.0 mg/ml. Among the tested isolates *C. albicans* ATCC10231 was found to contain a prophage which was successfully induced by mitomycin-C. The induced phage was isolated, purified and designated CAIP1. No prophages were detected neither in the other twenty six isolates of *C. albicans* nor the two isolates of *S. cerevisiae*.

The single plaques formed by the induced temperate phage were found to be circular turbid plaques with diameter of 2mm [Fig-1].

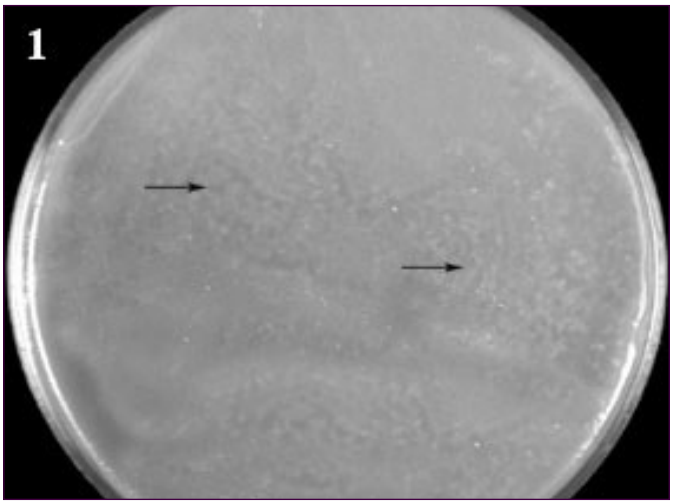

Fig. 1- Single Plaques of the induced phage isolate (CAIP1). Single plaques are indicated by arrows

Host Range of the Induced Phage Isolate

Susceptibility of each of the twenty seven *C. albicans* isolates and the two isolates of *S. cerevisiae* to the induced temperate phage (CAIP1) was tested. The obtained results indicate that temperate phage (CAIP1) was infectious to *C. albicans* ATCC 10231 among the isolates tested. The other twenty six *C. albicans* and two *S. cerevisiae* isolates were found to be resistant to the induced phage (CAIP1).

Particle Size and Morphology of the Induced Phage

The particles of the isolated temperate phage (CAIP1) were negatively stained with phosphotungstic acid and examined by transmission electron microscopy. As shown in [Fig-2] the phage was found to be hexagonal icosahedral particles of 78 nm in diameter.

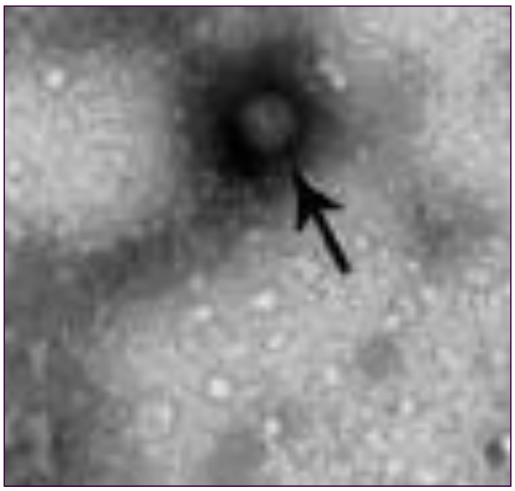

Fig. 2- Electron micrograph of the induced phage (CAIP1) negatively stained with (2%) phosphotengestic acid at pH 6.8. The phage particle is indicated by arrow

Cytopathic Effects of the Induced Phage on *C. albicans* Cells

The electron micrograph of the ultrathin sections of healthy *C. albicans* cells [Fig-3] (A&B) show that the cells have normal cell wall thickness, normal size and circular shape of 5 µm in diameter. Whereas, the phage-infected cells of *C. albicans* ATCC 10231 exhibited changes in cell size (diameter about 2.4µm) and slight malformations on their surfaces as shown in [Fig-4](A,B&C). In the late phase of infection, cytoplasmic coagulation, lysis of the cell wall and release of the induced phage particles were also observed [Fig-5].

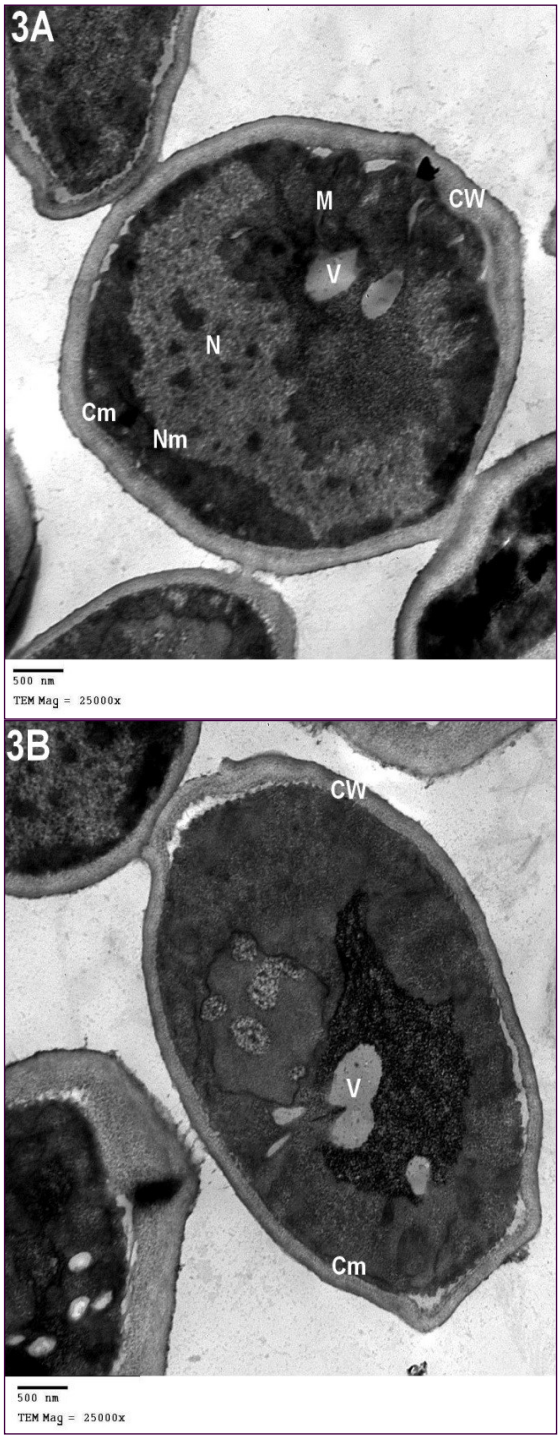

Fig. 3(A&B)- Healthy cells of *C. albicans* ATCC 10231 isolate

CW= Cell wall, V = Vacuole, N = Nucleus, Nm = Nuclear membrane, Cm = Cytoplasmic membrane, M = mitochondria.

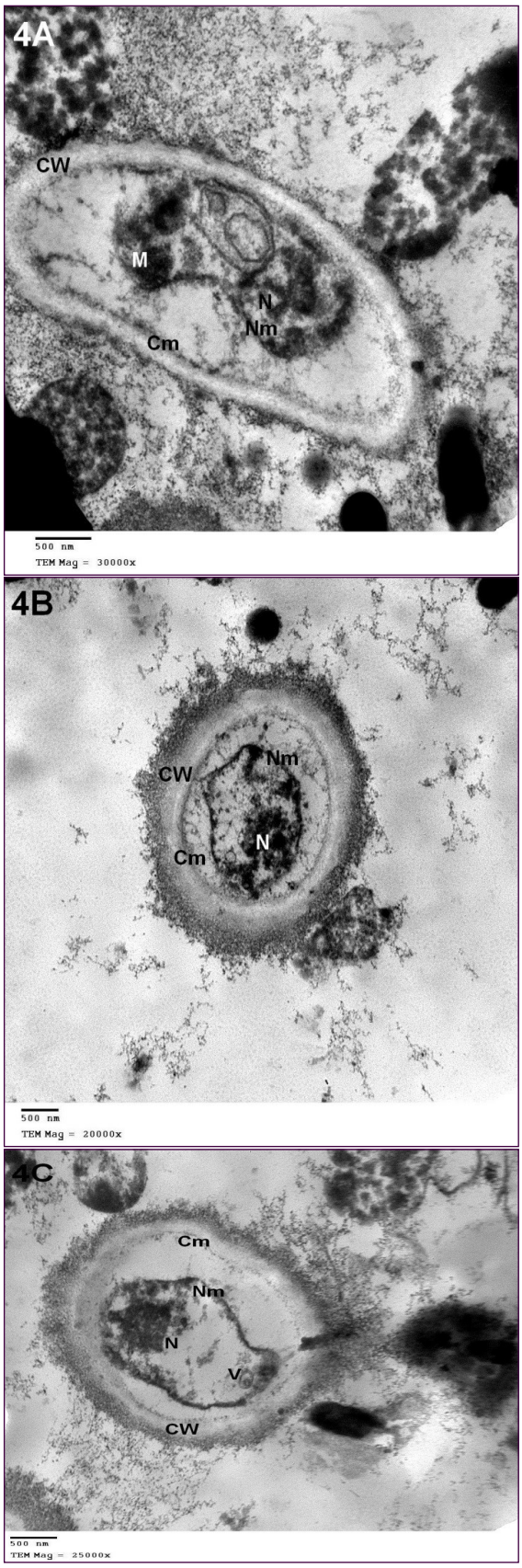

Fig. 4 (A,B&C)- Electron micrographs of infected cells of *C. albicans* ATCC10231 isolate

CW= Cell wall, V = Vacuole, N = Nucleus, Nm = Nuclear membrane, Cm = Cytoplasmic membrane, M = mitochondria.

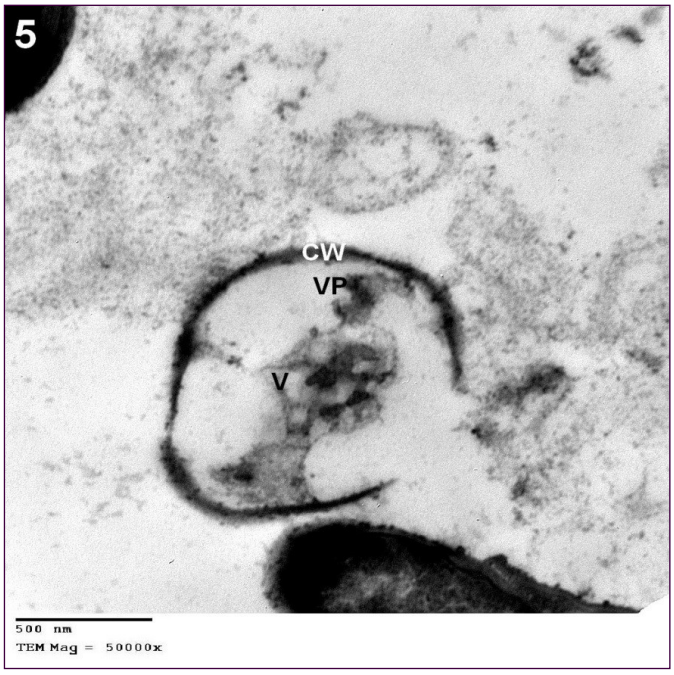

Fig. 5- Electron micrographs of *C. albican* ATCC10231 infected cells with endogenous virus (CAIP1) showing the virus particles and lysis of cell wall to emerge the virus particles

CW = Cell wall, V = Vacuole, VP = Virus particles

Discussion

In the present study a temperate phage was induced from *C. albicans* ATCC 10231 isolate using mitomycin-C at concentration of 1.0 mg/ml. The induced temperate phage was designated CAIP1.

This result is relatively in agreement when used the same concentration of mitomycin-C to induce a temperate phage from a strain of *Bacillus megaterium* [18]. They also noticed that there was a preferential inhibition of DNA synthesis in the lysogenic strain of *Bacillus megaterium* growing culture at exponential phase for the first 60-90 min. After 90 min., DNA synthesis recovered and exceeds RNA synthesis. The increase in DNA by lysogenic culture may be due to the production of the phage DNA.

The induced phage (CAIP1) formed circular turbid plaque of 2mm. in diameter. Similarly, observed cell lysis formed at 4-6 days in virus infected shake cultures of *Penicillium stoloniferum* (ATCC14586) in medium containing corn steep liquor [19]. Moreover, the *P. citrinum* and *P. variable,* showed lytic plaques developed when virus infected cultures were grown on lactose- peptone medium, although in a malt yeast peptone medium, this lysis did not occur [20].

Susceptibility of each of the twenty seven *C. albicans* isolates and the two isolates of *S. cerevisiae* to the induced temperate phage (CAIP1) was investigated qualitatively by the spot test. The temperate phage (CAIP1) was found to be of a narrow host range, since it was infectious to *C. albicans* ATCC 10231 among the isolates tested. Similarly, the host range of phages specific for *C. albicans* and *S. cerevisiae*. They found that the yeast phages are of narrow host ranges, since each phage was infectious only to its own host [21].

The induced yeast phage was examined in a transmission electron microscope. The phage is hexagonal icosahedral particles of 78 nm in diameter. According to the shape and size of the viral particles, this virus may be classified under family *Pseudoviridae*. The morphology of the virus particles was found to be similar to those of *Piricularia oryza* and *Rhizoctonia solani* isolated, but the diameters of these viruses are different since they are between 33-41 nm and 30 nm, respectively by Kovács, et al [22] and El-Araby [4].

Ultrathin sections of cells of *C. albicans* ATCC10231 isolate infected with the induced phage showed different cytopathic effects in the cell structure compared with the healthy one. The cytopathic effects include coagulation of cytoplasm, malformation of mitochondria and nuclear membrane, appearance of some virus particles and cell wall lysis. These results were relatively in agreement when examined the distribution of virus particles in thin sections of hyphae from several species of *Penicillium.* Young apical regions of hyphae were free of virus particles, obtained by Border, et. al. [23]. Moreover, the aggregation of virus particles and association of aggregates with membranes have been observed in thin sections of hyphae from *P. chrysogenum* [24,25], while in *P. cyaneofulvum* and *P. funiculosum* were observed by Border, et. al. [23] and in *S. cerevisiae* cells were showed by Border [26]. The virus reaches a high titer in host cells causing cellular aberrations [27]. The mycoviruses move intercellularly during cell division [19].

Conclusion

Generally, on the basis of the obtained results it can be concluded that, a temperate phage was induced from *candida albicans* ATCC10231 using mitomycin-C at concentration of 1µg/ml. The induced temperate phage produced circular turbid single plaques of 2mm in diameter. The induced temperate phage was of narrow host range. The phage was found to be hexagonal icosahedral particles of 78 nm. Ultrathin section of infected *C. albicans* cells showed cytoplasmic coagulation, malformation of mitochondria as well as nuclear membrane and finally lysis of the cell wall and release of the induced phage particles were observed.

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Conflicts of Interest: None declared.

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